Bacterial Enteric Pathogens: Shigella
Presentation to WHO Vaccine Advisory Committee
June 7 to 9, 2016
Presentation contents

- Introduction to the landscape
- Cellular candidates for oral delivery
- Subunit candidates for parenteral delivery
- Vaccine presentation for oral delivery in endemic settings
- Possibilities for combined ETEC/Shigella vaccines
- Summary
Shigella disease

- Four serogroups of *Shigella*
  - Flexneri (16 serotypes)
  - Sonnei (1 serotype)
  - Bodyii (19 serotypes)
  - Dysenteriae (15 serotypes)

- Symptoms
  - Diarrhoea (often containing blood or mucous)
  - Abdominal cramps and fever

- Major vaccine antigens
  - O polysaccharide of LPS
  - Outer membrane proteins
  - Type 3 secretion system proteins
Shigella vaccine development: TPP summary

<table>
<thead>
<tr>
<th>High Level TPP</th>
<th>Minimum</th>
<th>Optimistic</th>
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<tbody>
<tr>
<td><strong>Indication</strong></td>
<td>Immunization against Shigella</td>
<td>Immunization against Shigella</td>
</tr>
<tr>
<td><strong>Target population</strong></td>
<td>&gt;1 year of age</td>
<td>&lt;1 year of age</td>
</tr>
<tr>
<td><strong>Efficacy</strong></td>
<td>50% against severe disease</td>
<td>70% against severe disease</td>
</tr>
<tr>
<td><strong>Duration of Protection</strong></td>
<td>At least 2 years</td>
<td>At least 4 years</td>
</tr>
<tr>
<td><strong>Safety</strong></td>
<td>Similar to placebo</td>
<td>Similar to placebo</td>
</tr>
<tr>
<td><strong>Route of administration</strong></td>
<td>Oral or alternate route that induces mucosal immunity</td>
<td>Oral or alternate route that induces mucosal immunity</td>
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</tbody>
</table>
### Current Shigella vaccine candidates

#### Oral Administration
- TSWC—killed (PATH)
- WR VirG series live attenuated (PATH, WRAIR)
- CVD GuaBA series live attenuated (UMB; PATH)

#### Parenteral Injection
- Invaplex (WRAIR; PATH)
- GMMA (SBVGH)

#### Clinical Candidates
- CVD GuaBA mutants expressing ETEC antigens (UMB)

#### Preclinical Candidates
- DB Fusion (PATH)
- Outer Membrane Vesicles (Univ. Navarra)
- 34 kDa OMP (Niced)
- ShigETEC (EVELIQURE)
- Ty21a expressing Shigella LPS (CombiVax)
- Truncated Shigella (IVI, PATH)
Cellular candidates for oral delivery
Trivalent Shigella killed whole cell (TSWC)

- Formalin-inactivated *S. flexneri* 2a, *S. flexneri* 3a, and *S. sonnei*

- Expected coverage from trivalent vaccine about 80%

- Safe and immunogenic in man when given up to $10^{11}$ vp

- Challenge trial with *S. flexneri* 2a prototype in 4Q2016

- Phase 1 trial with complete vaccine alone and co-administered with ETVAX 1Q2017
Live, attenuated *Shigella* vaccine: VirG series

- WRSS1 is the *S. sonnei* component of a multivalent vaccine being developed at Walter Reed Army Institute of Research (WRAIR)
- Attenuated by VirG deletion which limits cell-to-cell spread of bacteria
- Safe and immunogenic in adult volunteers
- Currently being evaluated at the iccdrb in adults, 5- to 9-year olds and 12- to 23-month old children
  - 5-9 yo cohort completed 1Q2016
  - 12-23 mo cohort begins 3Q2016
- WRSs2 and WRSs3 are Shet2-1 and Shet2-2 + MsbB2 Mutants for greater attenuation: in Phase 1 trial to be completed 4Q2016
Live, attenuated *Shigella* vaccine: GuaBA series

- **Basis of attenuation:**
  - *guaBA*: chromosomal operon regulating biosynthesis of guanine nucleotides.
  - *set*: chromosomal gene encoding *Shigella* enterotoxin 1 (ShET1)
  - *sen*: plasmid gene encoding ShET2

- **CVD 1208S-GMP** is both well-tolerated and elicits immune responses that have been correlated with protective efficacy.

- Vigorous IgA ASC responses to *S. flexneri* LPS were detected in 10 out of the 12 vaccine recipients in $10^8$ cfu cohort in the absence of reactogenicity events.

- That some subjects mounted ASC responses after multiple doses suggests that the multi-dose regimen may be effective in recruiting additional responders/responses.

Data courtesy Karen Kotloff, CVD
CVD hybrid *Shigella*-ETEC vaccine strategy

- Antigen expression confirmed by quantifying transcripts
- by RT-PCR and demonstrating protein expression by Western blot
- Strain tested in guinea pigs
  - 8/8 had robust titers serum IgG and mucosal IgA to *S. flexneri* 2a LPS and ETEC CFA/I
  - 4/8 responded to LTB
  - Immune serum inhibited hemagglutination with WT ETEC H10407
  - 8/8 animals protected against wild type *S. flexneri* 2a (2457T) challenge via Sereny test
- Team may develop consortium to make GMP lot of prototype and conduct clinical evaluations
Live, attenuated *Shigella* vaccine (ShigETEC): IpaBC series

- *S. flexneri* 2a (2457T) attenuated by deletion of IpaBC tandem from Ipa gene cluster of the large invasion plasmid; non-invasive strain with low/no reactogenicity
- O-antigen component of LPS removed to enable serotype-independent protection (in mouse lung model); antibody induced against as yet undefined cell surface antigens
- Expression of LTB/subunit—ST toxoid fusion protein elicited both LT and ST antibody responses in mice
- Western blot analyses of sera from vaccinated animals revealed considerable cross reactivity among serotypes
- Current status:
  - GMP manufacture to be initiated in Q3 2016
  - Phase 1 study completion in adults Q4 2017 (may move into DA following phase 1)
  - Phase 2 efficacy data from human challenge studies Q4 2018

Data courtesy of Gabor Somogyi, EveliQure
TY21a expressing *Shigella* O-antigens

- *Shigella* O-antigen gene clusters inserted into Ty21a chromosome

- Mice immunized IN with Ty21a-Ss
  - produced IgG antibodies against both pathogens
  - were protected against a lethal IN *S. sonnei* 53G infection

Data courtesy of Dennis Kopecko, CombiVax
Truncated mutant

• Targets OMP for universal Shigella vaccine

• Mutant strains have only one unit of O-antigen

• Shortening layer of LPS allows OMP to be better exposed

• Three IN immunizations with live or formalin-inactivated mutant whole cells of S. flexneri 2a induced cross protection against S. flexneri 2a, S. flexneri 6, and S. dysenteriae 1 in mouse pneumonia model

Data courtesy of Jae-Ouk Kim, IVI
Subunit candidates for parenteral delivery
Prospects for mucosal immunity from parenteral vaccination: LT-based adjuvants direct fecal anti-adhesin response

- Adult female BALB/c mice
- Vaccination on days 0, 14, 28
- Titers shown, day 42
- All LT forms given ID, 0.1 μg
- mLTL given IN, 1.5 μg

Data courtesy of S. Savarino, NMRC
Artificial invaplex (InvaplexAR)

- **Shigella Invasin Complex** (Invaplex)
  - Assembled from recombinant IpaB, IpaC and wild-type LPS using molar ratios established with highly purified native Invaplex
  - Retains biological activity associated with native Invaplex, but is more immunogenic
  - Well-defined product
  - Customizable constituents
  - Manufactured and assembled under cGMP
  - Phase 1 clinical evaluation delivered intranasally: clinical phase completed May 2016

Data courtesy of Rob Kaminski, WRAIR
Purpose of using “detoxified LPS”

- Wild-type LPS may be reactogenic when administered via a parenteral route
- Lipid A moiety of the LPS responsible for endotoxic activity
- *Shigella* lacking late acyl transferases (ΔmsbB) have been constructed

Genetically-attenuated lipid A mutants are under-acylated and less endotoxic:

- *in vitro* (monocyte activation test)
- *in vivo* (reduced reactogenicity)

Data courtesy of Rob Kaminski, WRAIR

(d’Hauteville et al., J Immunol. 2002, 5240-51)
Detoxified invaplexAR (InvaplexAR-Detox)

- LPS isolated from ΔmsbB Shigella mutants produce deacetylated (tetra and penta-acylated) lipid A
- Deacetylated LPS induces LESS pro-inflammatory cytokine release from macrophages *in vitro*
- Lower reactogenicity (edema, induration, erythema) as compared to InvaplexAR after ID vaccination of mice
- Induces same levels of *Shigella*-specific antibodies in mice and guinea pigs as compared to InvaplexAR
- cGMP manufactured
- Evaluation in Phase 1 trials using parenteral route targeted for 1Q2017 if funded

Data courtesy of Rob Kaminski, WRAIR
In vivo produced glycoconjugates

- Complete *in vivo* *E. coli* process
- Homogeneity/reproducibility
- Easy to characterize
- Mild coupling conditions
- Folded carrier protein/glycan

Data courtesy of Cristina Alaimo, LimmaTech
The bioconjugation platform

- **Key Features**
  - Target Product Profile is a **pentavalent conjugate**
    - *S. dysenteriae* and *S. flexneri 2a* conjugates safe and immunogenic in phase I studies
    - *S. flexneri 3a, 6* and *S. sonnei* conjugates tested in preclinical studies
  - 2 doses IM for infants and children; 1 dose adult populations

- **Advantages over traditional conjugation**
  - Enables development of vaccines for a range of indications, which are difficult to produce with chemical conjugation
  - Versatility across pathogens and conjugates
  - Natural conjugation, no chemicals needed, preservation of antigens
  - Simplicity of recombinant manufacturing, resulting in faster development time, and cost reduction
  - Highly reproducible process resulting in a homogeneous product and batch-to-batch consistency

Data courtesy of Cristina Alaimo, LimmaTech
Flexyn2a (2a O-antigen of S. flexneri-EPA)

- Tested for safety and immunogenicity in a placebo controlled phase I trial, conducted in 2015 at the NMRC
- Currently tested for protection against shigellosis in a Phase 2b challenge study, conducted at the JHU in Baltimore (study results by end of 2016)

Data courtesy of Cristina Alaimo, LimmaTech
Synthetic carbohydrates vaccine for *Shigella*

- Homogeneous, well defined oligosaccharides (OS) offer alternatives to conjugates of detoxified LPS
  - LPS detoxification step can result in loss of immunogenicity
  - specific OS can be better immunogen than native polysaccharide
- SF2a-TT15
  - Induced Ab recognizing live Sf2a bacteria
  - Recognized by sera from Sf2a infected individuals
  - Optimum OS selected on basis of immunogenicity testing and protection in mice.
- Phase 1 initiated 2Q2016 (10 and 2 mg/dose ± alum 3 doses at 3 week intervals

Data Courtesy of Armelle Phalipon, Institut Pasteur
Generalized Module for Membrane Antigens (GMMA)

- Pure outer membrane buds by genetic engineering → efficacy & affordability of whole cell vaccine without the side effects
- Remove / modify toxic components
  - Lipid A of LOS or LPS
- Delete unwanted antigens
  - surface polysaccharides, capsule
- Add new antigens
  - over express homologous antigens
  - add heterologous antigens

GMMA *Shigella* vaccine

- Studies with *S. sonnei* prototype in healthy European adults completed
- Safe at all doses when delivery by the IM, IN and ID routes
- High serum anti-LPS IgG responses when administered by IM route
- Ongoing activities: Adult trial in Kenya for safety and immunogenicity
- Next steps:
  - Age de-escalation trials in Kenya to determine balance of safety and immunogenicity of GMMA-based vaccine in younger age groups (2017)
  - Human challenge trial to evaluate clinical protection (2017+)

- 4-valent GMMA formulation (*S. sonnei*, *S. flexneri* 2a, 3a and 6) tested in mice. No immunologic interference observed in the anti-OAg serum response of the multivalent formulation compared with single GMMA
- Next steps:
  - GMP production of *S. flexneri* GMMA drug substance (2017)
  - First in human safety and immunogenicity of 4-valent vaccine (2019)

Data courtesy of Laura Martin, GSK
DB fusion

- Based on TTSS encoded in invasion plasmid in all virulent *Shigella* spp.
- IpaB and IpaD elicit strong immune responses in mice:
  - High serum IgG and IgA titers
  - Specific ASCs in spleen, BM, and nasal tissue
  - IFN-γ secreting cells
- IpaB and IpaD conferred protection against different serotypes using
  - a lethal pulmonary challenge model in mice
- IpaB and IpaD fused into one protein with similar demonstration of
  - immunogenic and protective properties
- Phase 1 trial with material delivered id with dmLT to begin in 3Q2017
- Phase 2b challenge to follow based on successful phase 1

Data courtesy of Wendy Picking, OSU
Protection following ID vaccination with DB Fusion

Data courtesy of Wendy Picking, OSU
34 kDa Protein (OmpA) of S. flexneri 2a

- Oral immunization with heat killed *S. flexneri* 2a protects against homologous challenge
- OMP identified as principal antigens in these preparations
- Of these the gel cut band of 34 kDa (OmpA) provides significant protection in rabbits against challenge
- This antigen is highly conserved on bacterial surface and evokes protective type-1 CMI
Outer membrane vesicles

- OMV naturally secreted into culture medium during the stationary phase of growth
- OMV are 40% LPS and contain antigens such as OmpA, OmpC/OmpF, IpaB, IpaC and IpaD
- Mice protected against IN challenge with homologous S. flexneri 2a after single immunization via intranasal, ocular or oral routes.
Vaccine presentation for oral delivery in endemic settings
Adaptation of basic oral presentation for pediatric use

Present endemic pediatric formulation
≈14-24 ml

1/8, ¼, ½ or full dose

2,5-10μg dmLT

Whole cell bulk + LCTB A

Buffer Sachet

10 ml H₂O

Next stage endemic pediatric formulation
≈3 ml

TBD, approx 0,75 ml

TBD, approx 2,25 ml

TBD, approx 0, 23 g

Whole cell bulk

= 2,25 ml H₂O

Buffer Sachet LCTBA + dmLT

PATH
Streamlined presentations for pediatric use

With buffer

- Frangible seal
- Water
- Buffer salts
- Buffer + dmLT

OR

- Frangible seal
- Cell suspension
- dmLT

Without buffer
Possibilities for combined ETEC/Shigella vaccines
**Current ETEC and *Shigella* vaccine candidates**

<table>
<thead>
<tr>
<th>Oral Administration</th>
<th>Parenteral Injection</th>
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<tbody>
<tr>
<td><strong>ETEC</strong></td>
<td><strong>Shigella</strong></td>
</tr>
<tr>
<td>• ETVAX – killed (PATH; SBH)</td>
<td>• TSWC – killed (PATH)</td>
</tr>
<tr>
<td><strong>Shigella</strong></td>
<td></td>
</tr>
<tr>
<td>• ACE527 live attenuated (PATH; NVSI)</td>
<td>• WR VirG series live attenuated (PATH, WRAIR)</td>
</tr>
<tr>
<td></td>
<td>• CVD GuaBA series live attenuated (UMB; PATH)</td>
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<tr>
<td><strong>ETEC</strong></td>
<td><strong>Shigella</strong></td>
</tr>
<tr>
<td></td>
<td>• FTA (Sanofi; PATH)</td>
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<tr>
<td></td>
<td>• Invaplex (WRAIR; PATH)</td>
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<tr>
<td><strong>Preclinical Candidates</strong></td>
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<td>• CVD GuaBA mutants expressing ETEC antigens (UMB)</td>
<td>• Ty21a expressing <em>Shigella</em> LPS (CombiVax)</td>
</tr>
<tr>
<td>• ShigETEC (EVELIQUE)</td>
<td>• MEFA (KSU, PATH)</td>
</tr>
<tr>
<td>• ZH9 typhoid--LT/ST toxoid (Prokarium)</td>
<td>• LT/ST Fusion/conjugate (ENTVAC Consortium, PATH)</td>
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<tr>
<td></td>
<td>• DB Fusion (PATH)</td>
</tr>
<tr>
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<td></td>
<td>• 34 kDa OMP (Niced)</td>
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</table>

*Current combination ETEC-Shigella candidates are enclosed in boxes*
Considerations for combined enteric vaccines

**Parenteral Administration**

- Novel regimen needed to induce mucosal immunity in addition to only systemic immunity
- Not needle free
- Not susceptible to negative effects of enteropathy

**Options**

- Combination into single vial critical
  - co-mixing presents compatibility challenges
  - conjugation may simplify combination
  - may present IP issues

Co-formulation for parenteral subunit vaccines
Considerations for combined enteric vaccines

**Oral Administration**

- Induces systemic and mucosal immunity essential for protection against many of the enteric pathogens
- Needle-free
- Susceptible to negative effects of enteropathy
- May require buffer to protect against antigen denaturation

**Options**

- May be co-administered rather than co-formulated
Options for developing combined oral vaccines using ETVAX and TSWC

- IP issues—Avoid mixing products of 2 companies
- License product when it is ready rather than holding
- Multi-tube approach compatible with delivery via oral route
- Promotes flexibility in immunization regimen—may not be limited to a particular candidate
Summary
Progress is being made towards *Shigella* vaccine development

- New cellular and subunit vaccine candidates under development
- Conserved protein antigens may be shown useful in vaccines thereby avoiding serotype-specific immunity
- Though important as stand-alone vaccines, many of these vaccine candidates lend themselves to inclusion in combination vaccine strategies:
  - TSWC + ETVAX
  - GuaBA *Shigella* expressing ETEC colonization antigens and toxoids
  - Conjugate of O-PS and protein antigens can cover multiple pathogens
- New adjuvant (dmLT) may enhance mucosal response to oral and parenteral vaccines.
- Work needed to develop more practical pediatric presentations for use in endemic settings
# Current status of Shigella vaccine candidates

<table>
<thead>
<tr>
<th>Clinical Status</th>
<th>Candidates</th>
<th>Total</th>
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</thead>
<tbody>
<tr>
<td>Active (in clinical trials)</td>
<td>TSWC, VirG, GMMA, Bioconjugate, Synthetic glycoconjugate</td>
<td>5</td>
</tr>
<tr>
<td>Inactive (passed phase 1, no further work scheduled)</td>
<td>GuaBA</td>
<td>1</td>
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<tr>
<td>Pending (Phase 1 trial anticipated)</td>
<td>GuaBA-ETEC, ShigETEC, Ty21a-Shigella, Detox. Invaplex&lt;sub&gt;AR&lt;/sub&gt;, DB Fusion</td>
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<tr>
<td>Preclinical (Clinical feasibility still to be determined)</td>
<td>Truncated mutant, OMV, 34 kDa protein</td>
<td>3</td>
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<tr>
<td><strong>Total</strong></td>
<td></td>
<td><strong>14</strong></td>
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</table>
Assessment of WHO role for Shigella vaccines 2016-2018

• Clinical trial design and endpoints of trials
• Preferred product characteristics; TPP
• Assessment of laboratory assays
  • qPCR vs. culture;
  • Immunology assays; correlates of protection