Vaccines against Enteric Bacteria:
Shigella and ETEC

Vaccinology Course 2017

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University of Maryland School of Medicine
Epidemiological settings where a vaccine might be useful

- **Endemic diarrhea**
  - Major cause of morbidity and mortality among children in developing countries

- **Traveler’s diarrhea**
  - Afflicts 10-40% of travelers to developing regions – ETEC is leading cause
  - Soldiers deployed to endemic countries can be quite debilitated by *Shigella* infection

- **High risk settings**
  - Day care centers, institutionalized settings (e.g., prisoners)

- **Pandemic and epidemic diarrhea**
  - *Shigella dysenteriae* type 1
The Global Enteric Multicenter Study (GEMS)

- A 3-year, matched case/control study of moderate-to-severe diarrhea (MSD) among children <5 years of age residing in censused populations at 7 sites in sub-Saharan Africa and S Asia:
  
  - Basse, Gambia
  - Bamako, Mali
  - Nyanza Province, Kenya
  - Manhiça, Mozambique
  - Kolkata, India
  - Mirzapur, Bangladesh
  - Karachi, Pakistan

Cases = 9,439
Controls = 13,129
Pathogen-specific incidence per 100 child years, by age stratum

0-11 months
- Rotavirus
- Cryptosporidium
- ST or ST/LT-ETEC
- Shigella spp.
- Adenovirus 40/41
- Aeromonas spp.
- C. jejuni
- tEPEC
- Norovirus GII
- V. cholerae O1
- NT Salmonella
- E. histolytica

12-23 months
- Rotavirus
- Shigella spp.
- Cryptosporidium
- ST or ST/LT-ETEC
- Aeromonas
- Norovirus GII
- V. cholerae O1
- Adenovirus 40/41
- EAEC
- tEPEC
- NT Salmonella

24-59 months
- Shigella spp.
- Rotavirus
- C. jejuni
- V. cholerae O1
- ST or ST/LT-ETEC
- Aeromonas spp.
- Sapovirus
- E. histolytica
- Norovirus GII
- NT Salmonella
- Cryptosporidium

Attributable Incidence per 100 child-years and 95% confidence limits
Role of ETEC and *Shigella* in Travelers’ Diarrhea

The role of ETEC and *Shigella* in post-infectious complications such as irritable bowel syndrome and reactive arthritis is also a consideration.

% troops sick in quarters or incapacitated

<table>
<thead>
<tr>
<th>Author (year)</th>
<th>Campylobacter</th>
<th>ETEC</th>
<th>Shigella</th>
<th>Other</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hyams (1991)</td>
<td>--</td>
<td>21%</td>
<td>64%</td>
<td>--</td>
</tr>
<tr>
<td>Cohen (2001)</td>
<td>--</td>
<td>--</td>
<td>56%</td>
<td>27%</td>
</tr>
<tr>
<td>Walz (2001)</td>
<td>--</td>
<td>--</td>
<td>92%</td>
<td>46%</td>
</tr>
<tr>
<td>Sanders (2002)</td>
<td>47%</td>
<td>--</td>
<td>--</td>
<td>27%</td>
</tr>
<tr>
<td>Monteville (2006)</td>
<td>--</td>
<td>26%</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Porter (2010)</td>
<td>50%</td>
<td>18%</td>
<td>--</td>
<td>20%</td>
</tr>
</tbody>
</table>

Emerging concern of antibiotic resistance: *Shigella* spp.


FROM M RIDDLE
**Emerging concern of antibiotic resistance: ETEC**

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>ETEC 1994-97 %Resistant</th>
<th>ETEC 2001-04 %Resistant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ampicillin</td>
<td>43</td>
<td>53</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>57</td>
<td>59</td>
</tr>
<tr>
<td>TMP/SMX</td>
<td>50</td>
<td>67*</td>
</tr>
<tr>
<td>Nalidixic acid</td>
<td>6</td>
<td>22*</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>1</td>
<td>8*</td>
</tr>
</tbody>
</table>

** High percentage of resistance to quinolones in ETEC isolated from travelers to North Africa and India

SHIGELLA VACCINES
## Clinical shigellosis in developing countries

### Acute illness:
- Fever
- Diarrhea
- Dysentery
- Tenesmus
- Abdominal Cramps
- Vomiting

### Complications:
- Dehydration
- Seizures
- Sepsis (rare)
- Toxic megacolon
- Metabolic derangement
- Rectal prolapse
- Intestinal perforation
- Reactive arthritis
- Hemolytic-uremic syndrome
- Persistent diarrhea
- Malnutrition
# Shigella serotypes and subtypes (ST)

<table>
<thead>
<tr>
<th>Group</th>
<th>No. ST</th>
<th>Epidemiology</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. sonnei</em></td>
<td>Single</td>
<td>Endemic – All</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Most common: high resource co.</td>
</tr>
<tr>
<td><em>S. flexneri</em></td>
<td>15</td>
<td>Endemic – low resource co.s</td>
</tr>
<tr>
<td><em>S. dysenteriae</em></td>
<td>15</td>
<td>Type 1 → Pandemics</td>
</tr>
<tr>
<td><em>S. boydii</em></td>
<td>20</td>
<td>Uncommon</td>
</tr>
</tbody>
</table>
Approach to Shigella vaccine development

- Major vaccine antigens
  - Serotype-specific:
    - O PS of LPS
  - Shared antigens
    - Outer membrane proteins
    - TTSS proteins, e.g., ipa
- Major virulence antigens that serve as basis for attenuation
  - Metabolic pathways (e.g., guaBA)
  - Cell-cell spread (virG)
  - Enterotoxins (Shet1 -sen and Shet2 set)
  - Shiga (stx, S. dysenteriae type 1)
  - Endotoxin (msbB)

Barry et al, Nat Rev Gastroent Hepatol 2013
Is an Effective Shigella Vaccine Feasible?

**Infection-Derived Immunity**

Age-specific rates of *Shigella* isolation from persons with diarrhea admitted to Ubon Provincial Hospital, Thailand, Jan - June 1987

- **S. dysenteriae 1**
- **S. flexneri**
Baseline S. sonnei O Antibody correlates with protection: Israeli Soldiers

<table>
<thead>
<tr>
<th>Baseline Titer</th>
<th>Shigellosis Attack Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;1:10</td>
<td>21/129 (24%)</td>
</tr>
<tr>
<td></td>
<td>(p=0.002)</td>
</tr>
<tr>
<td>≥1:10</td>
<td>4/61 (6.5%)</td>
</tr>
</tbody>
</table>

(Cohen et al. 1991)
### Infection-Derived Type-Specific Immunity

<table>
<thead>
<tr>
<th>Source</th>
<th>Type of Protection</th>
<th>% Protection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monkeys*</td>
<td>Homologous</td>
<td>100%</td>
</tr>
<tr>
<td></td>
<td>Heterologous</td>
<td>0%</td>
</tr>
<tr>
<td>Volunteers**</td>
<td>Homologous</td>
<td>70-80%</td>
</tr>
<tr>
<td>Cohort in Endemic Area***</td>
<td>Homologous</td>
<td>72%</td>
</tr>
</tbody>
</table>

Correlates of Immunity Unknown, but Responses Correlate with Protection (PE) vs. Wild Type Challenge in Volunteer Challenge Studies

<table>
<thead>
<tr>
<th>Vaccine</th>
<th>Dose (cfu)</th>
<th>% Anti-LPS Response</th>
<th>IgA ASC (GM)</th>
<th>IgG Ab</th>
<th>Efficacy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild type</td>
<td>$10^3$</td>
<td>92% (239)</td>
<td>50%</td>
<td>70%</td>
<td></td>
</tr>
<tr>
<td>EcSf2a-2</td>
<td>$10^9$</td>
<td>100% (59)</td>
<td>53%</td>
<td>48%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>$10^8$</td>
<td>100% (16)</td>
<td>19%</td>
<td>27%</td>
<td></td>
</tr>
<tr>
<td>SC602</td>
<td>$10^4$</td>
<td>58% (26)*</td>
<td>10%</td>
<td>50%</td>
<td></td>
</tr>
</tbody>
</table>

*43 in those challenged
## Protective Efficacy of Sm(d) Shigella Vaccines in Field Trials

<table>
<thead>
<tr>
<th>Parent:</th>
<th>2a</th>
<th>2a &amp; 3</th>
<th>1 &amp; 2a</th>
<th>3 &amp; sonnei</th>
</tr>
</thead>
<tbody>
<tr>
<td>#Subject:</td>
<td>675</td>
<td>278</td>
<td>3,624</td>
<td>3,663</td>
</tr>
<tr>
<td>Age:</td>
<td>Adults</td>
<td>Adults</td>
<td>2-8 yrs</td>
<td>2-8 yrs</td>
</tr>
<tr>
<td>Dose:</td>
<td>$10^{10}$</td>
<td>$10^{10}$</td>
<td>$10^{10}$</td>
<td>$10^{10}$</td>
</tr>
<tr>
<td>Efficacy:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-Clin</td>
<td>84-100%</td>
<td>85%</td>
<td>91%</td>
<td>82%</td>
</tr>
<tr>
<td>-Bact</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
</tbody>
</table>

*(Mel et al)*
How to choose vaccine serotypes
(The CVD cross-protection strategy)

- *S. flexneri* (66% of *Shigella* in GEMS)
  - 2a, 3a, and 6:
    - In GEMS, represent 40% (20%, 9%, and 11%) of isolates
    - In animals, these 3 serotypes cover/cross-protect all 15 serotypes

- *S. sonnei* (24% of *Shigella* in GEMS)
  - Major cause of endemic shigellosis in industrialized countries
  - Only 1 serotype exists

- *Sd1* (the cause of pandemic shigellosis)

[Livio 2014]
**Shigella live oral attenuated vaccines in clinical development**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Mutation</th>
<th>Phase</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. flexneri</em> 2a CVD 1208S</td>
<td>guaBA, sen, set</td>
<td>Phase 2</td>
<td>Kotloff</td>
</tr>
<tr>
<td><em>S. flexneri</em> 2a SC602</td>
<td>virG, iuc</td>
<td>Phase 1-2</td>
<td>Coster, Katz Rahman</td>
</tr>
<tr>
<td><em>S. sonnei</em> WRSS1</td>
<td>virG</td>
<td>Phase 1-2, children, dev co.</td>
<td>Kotloff Katz</td>
</tr>
<tr>
<td><em>S. sonnei</em> WRSS2, WRSS3</td>
<td>virG, senA, senB, msbB2</td>
<td>Phase 1-2</td>
<td>Barnoy Collins</td>
</tr>
<tr>
<td><em>S. dysenteriae</em> 1 WRSd1</td>
<td>virG, stxAB</td>
<td>Phase 1</td>
<td>McKenzie</td>
</tr>
<tr>
<td><em>S. flexneri</em> 2a WRSf2G11, 12, 15</td>
<td>virG, senA, senB, msbB2</td>
<td>Phase 1</td>
<td>Ranallo</td>
</tr>
</tbody>
</table>
Phase 1 trial of cGMP lot: 3 doses of $10^8$ cfu dose of CVD 1208S (n=12) or placebo (n=4).

- Well tolerated
- Excreted by 9 (75%) for several days, most after dose 1
- IgA/IgG anti-LPS ASC (67%), geometric mean 86.3, range 14-1500/$10^6$ PBMC.
  - One subject did not respond (also did not excrete) and one subject received only one dose
  - 2 subjects responded beyond dose 1
- IgA/IgG seroconversion (42%)
Live, attenuated *Shigella* vaccine: $\Delta$VirG series

- **WRSS1** - *S. sonnei* component of a multivalent vaccine developed at WRAIR
- $\Delta$*virG* limits cell-to-cell spread of bacteria
- Immunogenic and some reactions in US adults
- 40% efficacy in Thai adults
- Currently being evaluated at the iccdr,b in adults, 5- to 9-year olds and 12- to 23-month old children
- WRSs2 and WRSs3 are Shet2-1 and Shet2-2 + MsbB2 Mutants for greater attenuation: in Phase 1 trial.
S. flexneri 2a \( \Delta \text{virG} \) (intracellular spread) and \( \Delta \text{iuc} \) (iron scavenging)

~North American Adults~ SC602 (Sansonetti et al.)

<table>
<thead>
<tr>
<th>N</th>
<th>Dose (cfu)</th>
<th>Adverse Reactions</th>
<th>GM ASC</th>
<th>Protection vs. Challenge</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>(10^2)</td>
<td>0%</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>48</td>
<td>(10^4)</td>
<td>20%</td>
<td>26</td>
<td>All shigellosis = 50%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Severe = 100%</td>
</tr>
<tr>
<td>49</td>
<td>(10^6)</td>
<td>60%</td>
<td>154</td>
<td>-</td>
</tr>
<tr>
<td>18</td>
<td>(10^8)</td>
<td>67%</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Shed for mean of 10 days; 5% shed >4 weeks
GM = geometric mean; ASC = antibody secreting cells

(Coster 1999; Katz 2004)
Clinical Trials of $10^4$, $10^5$, $10^6$ cfu SC 602 in Matlab, Bangladesh

- Adults
  - 5 inpatients
    - Well-tolerated
    - Short term colonization after $10^6$ cfu
    - Seroresponse in 2 of 5 subjects after $10^6$ cfu
  - 68 outpatients
    - No reactogenicity or excretion
    - Rare immune response, not dose-related
    - No household transmission

- Children 8-10 years old
  - No reactions, excretion, or immune responses

- Children 1-3 years old (n=34)
  - No reactions, excretion, or immune responses
Other live oral Shigella vaccines in preclinical development

<table>
<thead>
<tr>
<th>Vaccine Description</th>
<th>Mutation</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ty21a expressing <em>Shigella</em> LPS (CombiVax)</td>
<td>NA</td>
<td>Kopecko</td>
</tr>
<tr>
<td>Truncated <em>Shigella</em> mutant (IVI)</td>
<td>?</td>
<td>Kim</td>
</tr>
</tbody>
</table>
**TY21a vaccine delivery platform: typhoid, shigellosis, and ETEC**

- **Typhoid fever**: Acid stabilized Ty21a to improve protective efficacy
- **Shigellosis**: *Shigella* O-antigen gene clusters inserted into Ty21a chromosome with acid resistance genes
- **ETEC diarrhea**: ETEC MEFA and LTB inserted stably into Ty21a chromosome

(Protein potential)
Truncated mutant: *Shigella* strains with shorter O-polysaccharide unit

- Increased exposure of conserved IcsP outer membrane protein on *Shigella* surface without affecting expression level
- In mice, immunization with mutant strains immunogenic
  - No clear evidence of heterologous immunity

Jae-Ouk Kim, IVI
## Parenteral Shigella subunit vaccines in clinical trials

<table>
<thead>
<tr>
<th>Construct</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Common protein-directed + LPS</strong></td>
<td></td>
</tr>
<tr>
<td>GMMA vesicles (ID, IM, IN) (SBVGH)*</td>
<td>OM + periplasmic proteins</td>
</tr>
<tr>
<td>Invaplex &amp; Invaplex$_{AR}$ (TTSS)</td>
<td>LPS + IpaB, C, D</td>
</tr>
<tr>
<td><strong>O-antigen based</strong></td>
<td></td>
</tr>
<tr>
<td>Chemical conjugate (LPS-rEPA)</td>
<td><em>S. flexneri</em> 2a</td>
</tr>
<tr>
<td></td>
<td><em>S. sonnei</em></td>
</tr>
<tr>
<td></td>
<td><em>S. dysenteriae</em> type 1</td>
</tr>
<tr>
<td>Bacterially-expressed glycoconjugate (Limmatech Biologics)*</td>
<td>Multivalent vaccine</td>
</tr>
<tr>
<td></td>
<td>conjugated to rEPA; to</td>
</tr>
<tr>
<td></td>
<td>date <em>S. dysenteriae</em></td>
</tr>
<tr>
<td></td>
<td>and <em>S. flexneri</em> 2a</td>
</tr>
<tr>
<td></td>
<td>constructs evaluated</td>
</tr>
</tbody>
</table>
**Generalized Module for Membrane Antigens (GMMA)**

- Pure outer membrane + periplasmic protein buds by genetic engineering
- High yield, cheap
- Intended to elicit cross-protection
- Add new antigens
  - over express homologous antigens, add heterologous antigens
- 4-valent GMMA formulation (*S. sonnei, S. flexneri* 2a, 3a and 6) immunogenic in mice
- *S. sonnei* prototype safe and immunogenic in phase 1; descending age and challenge trial start 2017

Detoxified invaplexAR (InvaplexAR-Detox)

- Detoxified LPS in a macromolecular complex with broadly conserved Ipa
- LPS isolated from $\Delta msbB$ Shigella mutants produce deacylated lipid A
- Deacylated LPS induces LESS pro-inflammatory cytokine release from macrophages in vitro
- Lower reactogenicity (edema, induration, erythema) as compared to Invaplex$_{AR}$ after ID vaccination of mice
- Induces same levels of Shigella-specific antibodies in mice and guinea pigs as compared to Invaplex$_{AR}$
- cGMP manufactured
- Evaluation in Phase 1 trials using parenteral route targeted

Data courtesy of Rob Kaminski, WRAIR
Israel Field Trial of S. sonnei chemically conjugated to rEPA in adults

Vaccine:
- Single IM injection
- 2.5-7 mo. follow-up

Efficacy:
- 74% (95% CI 28-100, p=0.006)
- 43% within 1-17 days after vaccination (p=0.04)

(Cohen DS et al. Lancet 1997;349:155)
## Field Trial of S. sonnei-rEPA in Israeli Children

*(Passwell J 2010, PIDJ)*

### 2 years after immunization

<table>
<thead>
<tr>
<th>Age</th>
<th>S. sonnei</th>
<th>Efficacy (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N=</td>
<td>Cases</td>
<td></td>
</tr>
<tr>
<td>1 yr – 2 yr</td>
<td>489</td>
<td>18</td>
<td>-5.4% (-104, 45.6)</td>
</tr>
<tr>
<td>2 yr – 3 yr</td>
<td>487</td>
<td>8</td>
<td>35.6% (-56.2, 73.4)</td>
</tr>
<tr>
<td>3 yr – 4 yr</td>
<td>378</td>
<td>3</td>
<td>70.8% (-5.3, 91.9)</td>
</tr>
<tr>
<td>Total</td>
<td>1404</td>
<td>29</td>
<td>27.0% (-17.8, 54.2)</td>
</tr>
</tbody>
</table>

- Overall efficacy 1-4 yr olds was not significant
- Only subanalysis for *S. sonnei* among 3-4 yr olds was significant
- Insufficient *S. flexneri* to assess efficacy
- Some suggestion of cross-protection among *S. flexneri* serotypes
Shigella bioconjugate vaccines

Key Features

- Target is a **pentavalent conjugate**
  - *S. dysenteriae* and *S. flexneri 2a* rEPA conjugates safe and immunogenic in phase I studies; no benefit from alum; single dose may be sufficient
  - *S. flexneri* 3a, 6 and *S. sonnei* conjugates tested in preclinical studies

Advantages over traditional conjugation

- Natural conjugation, no chemicals needed, preservation of antigens
- Simple recombinant manufacturing, faster and cheaper
- Highly reproducible process resulting in a homogeneous product and batch-to-batch consistency

Hatz; Riddle
Parenteral Shigella subunit vaccines in pre-clinical development

<table>
<thead>
<tr>
<th>Common protein-directed</th>
<th>Construct</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Purified Ipa proteins (DB fusion), +/- dmLT</td>
<td><em>S. flexneri</em> 2a IpaB + IpaD</td>
<td>Martinez-Becerra</td>
</tr>
<tr>
<td>Outer membrane vesicles (U Navarra)</td>
<td>OM + periplasmic proteins</td>
<td>Berlanda</td>
</tr>
<tr>
<td>14 kDa OMP</td>
<td>PSPP-1</td>
<td>Kim</td>
</tr>
<tr>
<td><strong>O-antigen based</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Synthetic glycoconjugate (Institut Pasteur, PATH)</td>
<td>O-antigen-TT + alum</td>
<td>Phalipon</td>
</tr>
</tbody>
</table>
DB fusion

- Based on TTSS encoded on invasion plasmid in all virulent *Shigella* spp.
- IpaB and IpaD fused into one protein
- Immunogenic and confer heterologous protection in mice against lethal pulmonary challenge
- Stalled/abandoned due to manufacturing problems

Data courtesy of Wendy Picking, OSU
Synthetic carbohydrates vaccine for Shigella

- Identify functional mimics of bacterial (OAg) or other PS antigens
- 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)

Armelle Phalipon, Institut Pasteur
ETEC VACCINES
Clinical ETEC

- Watery diarrhea
- +Vomiting
- +Fever
- In GEMS, associated with linear growth faltering and death in infants
Pathogenesis of Enterotoxigenic E. coli (ETEC): Mucosal Adherence & Enterotoxin production

- **Fimbria**

- **Toxins**
  - LT plus ST or ST only
  - Cause increased secretion, decreased absorption of water & ions
Colonization Factor Antigens

- CFA/I
- CFA/II family (CS1-3): express coli surface (CS) antigen 3, either alone with CS1 or CS2
- CFA/IV family (CS4-6): express CS6, either alone or with CS4 or CS5
- Other CS: 7, 12, 14, 17, 21
- Putative colonization factors: PCFO159, PCFO166, PCFO20
Which ETEC antigens are protective?

- ST is not immunogenic – trying to do better than nature
- LT confers short term protection
- Bovine anti-CFA immunoglobulin was protective in adult volunteers (Tacket et al)
- Volunteers were protected from heterologous challenge with shared CFA
- Nested case-control study of 397 Egyptian children < 3yrs to correlate serum ab levels to CFA and toxins vs. diarrhea
  - Found that serum anti-CFA/I correlates with protection from CFA/I-producing strains (Rao 2005)
"A vaccine meeting target coverage of 80% of ETEC strains globally would appear to require 7 to 8 CFs (CFA/I, CS1-3, CS4-6, and CS21) assuming the utilization of an LT toxin component."

Isidean et al. Vaccine 2011
Double mutant heat labile toxin (dmLT) - May facilitate enteric immunization

- Potent antigen and mucosal adjuvant.
- Increases humoral and cellular immune responses.
- Induces antibodies that neutralize LT enterotoxin activity.
- Effective by many routes; oral, sublingual, intramuscular, intradermal, TCI, etc.
- Can increase number of responders and facilitate dose sparing.

# Current ETEC Vaccine Landscape

## Oral

ETVAX inactivated (SBH, PATH)

## Parenteral

FTA (PATH, NMRC, Sanofi, IDRI)

### Clinical Candidates

- ACE527 live attenuated (PATH; NVSI)

### Preclinical Candidates

- CVD ΔguaBA mutants expressing ETEC antigens (UMB)
- ShigTEC (EVELIQURE)
- Ty21a expressing *Shigella* LPS and MEFA (Protein Potential)
- MEFA (KSU, PATH)
- LT/ST Fusion/conjugate (ENTVAC Consortium, PATH)
- Flagellin, EtpA, EatA, EaeH, YghJ
Oral Inactivated Whole Cell ETEC – Most advanced ETEC candidate

- Previous construct protected in the volunteer model but did not protect young children in Egypt (Savarino et al)
- New construct overexpresses CFA/I, CS3, CS5, and CS6 (total 10^{11} bacteria) and is adjuvanted with dmLT (10ug) and 1 ug LTB-CTB hybrid toxoid (ENTVAX)
- In Swedish adults
  - Met immunogenicity endpoints
  - CS6 most immunogenic with low dose dmLT (10 ug)
  - Memory responses evoked by boost 6-12 months
  - Some cross-reactive mucosal responses with non-vaccine CSs seen
<table>
<thead>
<tr>
<th>Phase 1/2: Descending age</th>
<th>Phase 1/2: Target age</th>
<th>Phase 2b: Travelers</th>
<th>Phase 2b: Infants in endemic area 2021-2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mirpur, Bangladesh:</td>
<td>Mirpur, Bangladesh:</td>
<td>Finish travelers</td>
<td></td>
</tr>
<tr>
<td>• Dose decending:</td>
<td>• Infants 6w, 10w,</td>
<td>to Benin</td>
<td></td>
</tr>
<tr>
<td>adults to infants 6-12</td>
<td>14w or 10w, 14w, 18w</td>
<td>• 18-65 yo</td>
<td></td>
</tr>
<tr>
<td>mo.</td>
<td>DBPC, dose-defining,</td>
<td>DBPC</td>
<td></td>
</tr>
<tr>
<td></td>
<td>+/- dmLT, efficacy</td>
<td>Two doses, 14 days</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3 doses, 28 days</td>
<td>apart</td>
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<td></td>
<td>apart</td>
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</tbody>
</table>
ACE527: Live, oral attenuated enterotoxigenic E. coli (ETEC) vaccine

- 3 strains, attenuated by deletions of aroC, ompC, and ompF.
- Expresses colonization factor antigen I (CFA/I), CS1, CS2, CS3, CS5, CS6, and heat labile enterotoxin subunit B (LT-B)
- Well tolerated at doses of up to $10^{11}$ CFU in a phase 1 trial in healthy adult volunteers
- Challenge:
  - 27% reduction in mod/severe diarrhea (primary endpoint)
  - Post hoc analysis: significantly reduced stool volume and shedding
- Repeat challenge with dmLT shows improved efficacy

(Darsey 2012)
### ACE527 ± dmLT: Efficacy Against Severe Diarrhea

**Vaccine Dose:** $10^{10}$ cfu of reconstituted lyophilized formulation (~$3 \times 10^9$ /strain).

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Severe Diarrhea</th>
<th>Protective Efficacy vs. Controls (P-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Yes (%)</td>
<td>No (%)</td>
</tr>
<tr>
<td>Controls</td>
<td>31</td>
<td>21 (68%)</td>
<td>10 (32%)</td>
</tr>
<tr>
<td>ACE527</td>
<td>13</td>
<td>7 (54%)</td>
<td>6 (46%)</td>
</tr>
<tr>
<td>ACE527 + dmLT</td>
<td>13</td>
<td>3 (23%)</td>
<td>10 (77%)</td>
</tr>
</tbody>
</table>

**Primary Endpoint:** Prevention of severe diarrhea defined as cumulative passage of more than 800 grams of grade 3 to 5 diarrhea stools for episodes beginning during the 120-hour observation period post-challenge.

Parental ETEC Fimbrial tip adhesins (FTAs)

(Savarino et al)

- The binding site domain
- Conserved across many CFAs: expect 3-5 FTA types needed
- Phase 1 clinical trials of transcutaneous and intradermal FTAs with mLT showed better performance ID
- Challenge study results are unpublished.
- Animal results suggest dmLT may improve immune responses
CVD's Shigella-ETEC vaccine

- An expression plasmid designed at CVD carried ETEC fimbrial and LThA2B antigens and the
- It also carried *hok-sok* balanced lethal system so plasmid loss would kill the bacterium, and a partitioning loci so when vaccine bacteria divide both daughter cells inherit a copy of the plasmid.

**Response to vaccine**
- No reactions seen after $10^7$ to $10^9$ cfu
- Mild reactions seen in 20% after $10^{10}$ cfu

- At the highest dose, 90% had IgA ASC responses to *S. flexneri* 2a LPS, achieving levels correlated with clinical protection
- However, beginning on Day 1, the excreted strains began to lose the plasmid, suggesting that mutations had occurred in the *hok-sok* balanced lethal system for plasmid stabilization
  - Result was that responses to ETEC antigens were uncommon
  - Future: chromosomal expression of ETEC antigens by the attenuated *Shigella* strain
CVD hybrid Shigella-ETEC vaccine strategy with chromosomal expression of ETEC genes in ∆guaBA attenuated Shigella strains

- Use as live vector or inactivated whole cell vaccine
- In 8 guinea pigs
  - All 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

*S. flexneri* 2a (CVD1208S-122) expressing ETEC CFA/I and LTA2B from chromosome

E Barry
# Current ETEC vaccine landscape

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Live, attenuated Shigella vaccine (ShigETEC)

- *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
- May soon enter clinical trials

(EveliQure)
Multi-epitope Fusion Antigen (MEFA) Vaccine for ETEC

- Approaches
  - Express dominant epitopes of CFAs in a single protein
  - Expresses non-toxic LTA LTB and ST in a single protein
  - MEFA vaccines stimulate neutralizing antibodies against each of these virulence antigens
- In piglets:
  - Safe
  - Reduced ileal colonization
- Easily purified at high yield; stable at room temp
- Readily used in combination vaccine as vector expressed (oral) or as parenterally injected antigen with other subunit vaccines

CFA/I - red, CS1 - orange, CS2 - yellow, CS3 - green, CS4 - blue, CS5 - purple, CS6 - cyan; CfaB backbone - grey

Data courtesy of Weiping Zhang, KSU
STToxoid

- 66 – 75% of all ETEC diarrheal cases caused by ST or LT-ST strains
- ST activates guanylate cyclase leading to fluid release
- In GEMS associated with more severe diarrhea in GEMS
- May suppress innate immune mechanisms so bacteria to colonize, proliferate and induce diarrheal disease.
- May also suppress the development of adaptive immunity against an ST or LT/ST expressing ETEC.

Development Challenges
- Detoxify ST for a safe vaccine
- Make ST immunogenic (ST toxin neutralizing Ab) through fusion/conjugation to carrier proteins.
Path Forward/Priorities

- If ETVAX works, hope is to develop a combination vaccine with KWC *Shigella* vaccine soon to be evaluated at CVD.
- Considerable debate about whether it is best to go forward with combination or single pathogen vaccines.
- The next 10 year promise a lot of activity in this area!!
What you might avoid until a vaccine is available..

Questions?

Photograph courtesy of J. Besser, MN Dept Health, M. Riddle