Bacterial Enteric Pathogens: *Enterotoxigenic Escherichia coli* (ETEC)

Presentation to WHO Vaccine Advisory Committee

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PATH
Presentation overview*

- Review of current ETEC and *Shigella* burden estimates – navigating transition
- Introduction to the ETEC vaccine landscape
- Cellular candidates for oral delivery
- Subunit candidates for parenteral delivery
- Summary and discussion

*Possibilities for combined ETEC/Shigella vaccines will be addressed by Dr. Walker in his Shigella vaccine presentation
Diarrheal morbidity remains high in infants and young children

- Diarrhea remains the second-highest killer of children due to infectious disease.
- Mortality has declined over the past four decades.
- Morbidity has not declined significantly, despite improvements in water and sanitation and benefits from oral rehydration therapy.
- The full, potential lifelong effects of enteric infections on human development, productivity, and chronic diseases is under-appreciated:
  - One-fifth of children worldwide (178 million) have stunted growth.
  - Early childhood enteric infections, with or without overt diarrhea, are predicted to account for 25 to 43% of this burden.
  - Burden and impact assessment remain influx

ETEC and *Shigella* disease burden in infants and children

- Nearly 2.7 billion cases of diarrheal disease every year, many with acute and chronic effects.
  - Globally, ETEC and *Shigella* are estimated to cause ~400 million episodes of diarrhea annually U5’s (WHO 2009).
  - GEMS confirmed that ETEC and *Shigella* are two of the top four pathogens causing moderate-to-severe diarrhea among U5’s in Africa and South Asia.
  - MAL-ED indicated that *Shigella* is an important pathogen at the community level and ETEC remains associated with hospitalization and chronic diarrhea.
  - Molecular re-analysis of the GEMS and MAL-ED data indicates ETEC and *Shigella* burden is higher than previously thought, particularly *Shigella* in 12-23m olds (~40%). *Observation has triggered burden re-assessments for both*

- ETEC and *Shigella* are also an important causes of illness among older children, with 100 million episodes in the 5 to 14 year age group; primarily in AFRO/SEARO.
- ETEC and *Shigella* are responsible for 13 million “disability-adjusted life years” (DALYs) annually and 1.8 million “years lived with disability” (YLDs) annually, combined.

*Estimated diarrhoeal deaths for U-5 children*

<table>
<thead>
<tr>
<th>Cause</th>
<th>% of total deaths (~600K)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rotavirus</td>
<td>37%</td>
</tr>
<tr>
<td>Other causes</td>
<td>40%</td>
</tr>
<tr>
<td>ETEC/Shigella</td>
<td>23%</td>
</tr>
</tbody>
</table>

*Estimated diarrhoeal deaths for U-5 children*
Global impact of ETEC and *Shigella* infections: IHME re-analysis

<table>
<thead>
<tr>
<th>Impact</th>
<th>ETEC</th>
<th><em>Shigella</em></th>
<th>Total</th>
<th>Diarrhea</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALL</td>
<td>59,000</td>
<td>74,000</td>
<td>74,000</td>
<td>166,000</td>
</tr>
<tr>
<td>UNDER 5</td>
<td>23,000</td>
<td>24,000</td>
<td>33,000</td>
<td>56,000</td>
</tr>
<tr>
<td>Total Cases</td>
<td>TBD</td>
<td>TBD</td>
<td>TBD</td>
<td>TBD</td>
</tr>
<tr>
<td>DALYs</td>
<td>3.1M</td>
<td>TBD</td>
<td>4.3M</td>
<td>TBD</td>
</tr>
<tr>
<td>YLDs</td>
<td>270,000</td>
<td>TBD</td>
<td>212,000</td>
<td>TBD</td>
</tr>
</tbody>
</table>

- TBD = To be determined
- *Shigella* is the 2nd leading cause of diarrheal mortality in all ages in GBD 2013 and 2015. Due to the moderate sensitivity of culture compared to qPCR case definition from the GEMS study which has big influences on estimates generated from the model. ETEC changes more modestly.
- BMGF-sponsored workshop on diarrheal disease burden estimates scheduled for 20 to 21 July 2016.

Data courtesy of IHME
Consequences of ETEC and *Shigella* infections – beyond direct measures

- Surveillance for ETEC and *Shigella* captures morbidity and mortality due to these pathogens.
- The unseen impact of these diseases have long term consequences for children and households.
- Cumulative effects of high burden drives further impoverishment at household level and lost schooling translates into reduced potential earnings (~5.8 trillion/yr. across 96 counties modeled).

*Size of triangles do not necessarily correspond directly to size of burden*

Source: Kotloff et al, 2012; Rheingans, R. et al., 2012; Victoria et al 2008
ETEC and *Shigella* are major contributors to stunting

- GEMS highlighted ETEC, *Shigella*, and *Cryptosporidium* as major contributors to the stunting burden.

- ETEC and *Shigella* episodes result in an estimated:
  - 2.6 million additional children with moderate stunting.
  - 2 million additional children with severe stunting.

- Checkley, et al. (2008) found increased odds of stunting for each five additional episodes of childhood diarrhea.
ETEC and *Shigella* burden compared to rotavirus

- Total DALYs from ETEC and *Shigella* (13 M) is similar to rotavirus DALYs due to acute under 5 diarrhea episodes (pre-vaccine).
- Greater than 50% of the ETEC and *Shigella* burden is directly from acute under 5 diarrhea.
- Once additional DALYs lost due to decreased cognition, increased risk of chronic diseases, and other long-term effects are added the ETEC and *Shigella* estimates, total burden may reach/surpass rotavirus estimates.
- Rotavirus infection has not been shown to be correlated with increase risk of stunting, cognition effects or other long-term effects.
Regional distribution of ETEC and *Shigella*: Regional hot spots by DALYS and Stunting

- Disease distribution may drive vaccine preferences.

- For some regions, a combined ETEC/*Shigella* vaccine will be important.

- Infection with ETEC/*Shigella* increases the number of children that are stunted, especially in regions with high rates of underlying stunting.

**Note:** DALY burden for stunting is directly attributed to ETEC and *Shigella* stunting. DALYs for greater than 5 year olds, cognition, and other long-term effects are not included in this figure.
• Intra- and inter-country disease heterogeneity is likely to exist.

• Possible for a country to contain areas encompassing the entire spectrum for ETEC and Shigella deaths/100,000 children.

• Understanding potential risk for death and disease is critical for country policymakers to decide where to introduce vaccines.

• Sub-national introduction of different vaccine products may be needed.
Potential impact of individual and combined vaccines: Mortality

- Blue bars = deaths averted between 2022 to 2050, if ETEC vaccine was deployed in 2022 and *Shigella/ETEC* combination vaccine deployed 5, 7, or 10 years later.

- Red bars = deaths averted between 2022 to 2050, if ETEC vaccine not deployed in 2022, but *Shigella/ETEC* combination vaccine deployed 5, 7, or 10 years later.

- Cost of waiting to introduce combined vaccine translates to fewer lives saved by 2050:
  - 5 yr delay: 64,000 additional deaths
  - 7 yr delay: 87,000 additional deaths
  - 10 yr delay: 117,000 additional deaths
Vaccine impact on cases: LiST model

• Existing LiST analysis by Johns Hopkins University provides insight into impact of stand-alone and combined vaccines on under 5 diarrheal cases.

• Analysis assumed vaccine efficacy against both ETEC and *Shigella* was 65% (80% strain coverage) and included herd effects for the combined vaccine (using cholera vaccine assumptions).

• Analysis assumed a lower proportion of diarrhea due to *Shigella* vs. ETEC, therefore may see an increase in number of cases averted with new burden estimates.
Introduction to the ETEC vaccine landscape
Enterotoxigenic E. coli (ETEC)

Major antigens:
- Fimbriae – *intestinal adherence*; >25 *types identified*
  - CFA/I
  - CFA/II CS1, CS2, CS3
  - CFA/IV CS4, CS5, CS6
  - Others (CS17, CS14, PCF071)
- Toxins – *causes diarrhea*; ~ 1/3 of strains each *toxin type*
  - LT LT, ST, ST/LT
- LPS – *important antigen*; >70 *types*
- Other targets – *aid in colonization and toxin delivery*
  - EtpA, EatA, Yghj, Ag 43, EaeH
- Symptoms and sequela
  - Watery diarrhea with subsequent dehydration
  - Stunting/chronic diseases
  - Potential functional bowel disorders in travelers (10-14%)
- Genome sequencing data indicate major ETEC lineages with distinct virulence profiles (CFs/toxins) are stable (1980 – 2011), globally transmitted, and isolated from both risk groups (travelers and children) (von Mentzer et al 2014, Nature Genetics).
Additional ETEC proteins may have vaccine potential

**Plasmid-based: ETEC-specific**

- **EtpA** – accessory adhesin on flagella; appears to also help in LT delivery.
- **EatA** – serine protease mucin components and helps deliver LT toxin; 80% homology with SepA of *S. flexneri*.

Recent study found more than half of ETEC strains examined made EtpA and/or EatA both contribute to anti-colonization immunity in the mouse model (Luo, Q et al., 2014 PloS one).

In mouse studies, an inverse relationship has been shown between anti-EtpA IgA and IgG fecal antibody levels and reduced colonization by ETEC (Fleckenstein et al, CVI 2016, In press).

**Chromosomal-based: May be present in commensals**

- **Yghj** – metaloprotease with mucinase activity.
- **EaeH** – adhesin that acts in concert with CF/CSs and with EtpA and EatA to help deliver LT.
- **Ag 43** – surface exposed autotransporter; contributes to anti-colonization intestinal immunity in mouse model; recognized by sera from experimental and natural infection.

# Current ETEC vaccine candidates

## Cellular - Oral Administration
- ETVAX – killed (PATH; SBH)

## Subunit - Parenteral Injection
- Fimbrial Tip Adhesin (FTA) (Sanofi; PATH)

## Clinical Candidates
- ACE527 live attenuated (PATH; NVSI)
- ShigETEC – Ph1 projected in 2017 (EVELIQuRE)
- CVD GuaBA mutants expressing ETEC antigens (UMB) – development consortium forming
- TyphETEC - ZH9 typhoid--LT/ST toxoid (Prokarium)

## Preclinical Candidates
- Multiepitope Fusion Antigen (MEFA) (U. Kansas; JHBSPH)
- LT/ST toxoids - Fusion/conjugate (ENTVAC Consort.)
- EtpA, EatA, EaeH, YghJ (WASHU and others)
## ETEC vaccine development: Target Product Profile summary

<table>
<thead>
<tr>
<th>High Level TPP</th>
<th>Minimum</th>
<th>Optimistic</th>
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</thead>
<tbody>
<tr>
<td>Indication</td>
<td>Immunization against ETEC</td>
<td>Immunization against ETEC</td>
</tr>
<tr>
<td>Target population</td>
<td>&lt;1 year of age (EPI schedule with boost at 9 mths.)</td>
<td>&lt;1 year of age (EPI schedule with boost at 9 mths.)</td>
</tr>
<tr>
<td>Efficacy</td>
<td>50% against severe diarrhoea</td>
<td>70% against severe diarrhoea</td>
</tr>
<tr>
<td>Duration of protection</td>
<td>2 years</td>
<td>4 years</td>
</tr>
<tr>
<td>Safety</td>
<td>Similar to placebo</td>
<td>Similar to placebo</td>
</tr>
<tr>
<td>Route of administration</td>
<td>Oral or alternate route that induces mucosal immunity</td>
<td>Oral or alternate route that induces mucosal immunity</td>
</tr>
</tbody>
</table>

ETEC Vaccine Development Landscape: Cellular Candidates
ETVAX/dmLT: Most advanced candidate in clinical development

- First-generation showed efficacy against severe travelers’ diarrhea, but not protective in Egyptian infants (impact on more severe illness could not be determined).

- WHO recommended that CF/CS content of vaccine be increased; add CS6 to formulation; and assess impact of adding adjuvant (dmLT) – all addressed in current candidate.
ETVAX clinical development plan outline: Two tracks

**Pediatric Indication**
- **OEV-122 - Phase I/II**
  - Dose-escalation, DA to 6 mos
  - Bangladesh
  - Ongoing
- **OEV-124 - Phase I/II**
  - Dose-escalation in 6 week old
  - Bangladesh
  - ~Q4 2016
- **OEV-128 - Phase III trial**
  - Infants – Bangladesh
  - Q3 2018
  - Efficacy and non-interference

**Safe/Immunogenic**
- Yes

**Traveler’s Indication**
- **OEV-123 - Phase IIB**
  - Adult travelers
  - Planning stage-2017
- **OEV-125 - Phase II**
  - Immunogenicity bridging
  - Adult travelers
- **OEV-126 - Phase III**
  - Confirmatory efficacy
  - Adult travelers
  - Licensure for traveler’s indication
- **Submission for Prequalification**

- **Safe/Immunogenic**
  - Yes

**OEV-121 – Phase 1**
- First-in-human
- Adults – Sweden -- 2012
Initial Phase 1 trial in Swedish adults exceeded expectations for safety and immunogenicity (OEV-121)

- Primary endpoint exceeded in all vaccine groups; highest frequency of responders to key antigens (83%) obtained with vaccine + low dose dmLT (10 ug).
- dmLT significantly improved B and T cell responses to CS6 component (lowest conc. in formulation).
- Follow-on memory study conducted by boosting subjects 12-23 months after their primary series - memory IgG cell to CF/CS and LTB detected; ALS response to all Ag’s accelerated and enhanced.
- Avidity of serum anti-LTB and ALS to LTB and CS3 significantly enhanced by boosting.

➢ T cell responses were also detected to CFA/I and CS5.
➢ Depletion experiments verified that IFN-γ and IL-17A was primarily produced by CD4+ T cells.
CF/CS mucosal responses better than expected: Induction of cross-reactive mucosal immune responses against CFs not included in the vaccine

Leach et al, 2015
**OEV-122: Age-descending, dose finding study – current status**

**PART A: 18-45 years, inclusive**

<table>
<thead>
<tr>
<th>Cohort</th>
<th>ETVAX</th>
<th>dmLT</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1</td>
<td>Full dose*</td>
<td>-----</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>Full dose* 10 µg</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Placebo</td>
<td></td>
<td>15</td>
</tr>
<tr>
<td></td>
<td><strong>Total</strong></td>
<td></td>
<td><strong>45</strong></td>
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</table>

*Full dose is one vial of ETVAX; for exact antigen concentration dose see Section 5.1*

**PART B: 24-59 months, inclusive**

<table>
<thead>
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<th>Cohort</th>
<th>ETVAX</th>
<th>dmLT</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>B1</td>
<td>¼ dose</td>
<td>-----</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>Placebo</td>
<td></td>
<td>10</td>
</tr>
<tr>
<td>B2</td>
<td>½ dose</td>
<td>-----</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>Placebo</td>
<td></td>
<td>10</td>
</tr>
<tr>
<td>B3</td>
<td>Full dose*</td>
<td>-----</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>Placebo</td>
<td></td>
<td>10</td>
</tr>
<tr>
<td>B4</td>
<td>Highest safe dose 2.5 µg</td>
<td>-----</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>Placebo</td>
<td></td>
<td>10</td>
</tr>
<tr>
<td>B5</td>
<td>Highest safe dose 5 µg</td>
<td>-----</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>Placebo</td>
<td></td>
<td>10</td>
</tr>
<tr>
<td>B6</td>
<td>Highest safe dose 10 µg</td>
<td>-----</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>Placebo</td>
<td></td>
<td>10</td>
</tr>
<tr>
<td></td>
<td><strong>Total</strong></td>
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<td><strong>150</strong></td>
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</table>

**PART C: 12-23 months, inclusive**

<table>
<thead>
<tr>
<th>Cohort</th>
<th>ETVAX</th>
<th>dmLT</th>
<th>(N)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C1</td>
<td>¼ dose</td>
<td>-----</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>Placebo</td>
<td></td>
<td>10</td>
</tr>
<tr>
<td>C2</td>
<td>½ dose</td>
<td>-----</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>Placebo</td>
<td></td>
<td>10</td>
</tr>
<tr>
<td>C3</td>
<td>Highest safe dose 2.5 µg</td>
<td>-----</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>Placebo</td>
<td></td>
<td>10</td>
</tr>
<tr>
<td>C4</td>
<td>Highest safe dose 5 µg</td>
<td>-----</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>Placebo</td>
<td></td>
<td>10</td>
</tr>
<tr>
<td></td>
<td><strong>Total</strong></td>
<td></td>
<td><strong>100</strong></td>
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</table>

**PART D: 6-11 months, inclusive**

<table>
<thead>
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<th>Cohort</th>
<th>ETVAX</th>
<th>dmLT</th>
<th>(N)</th>
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</thead>
<tbody>
<tr>
<td>D1</td>
<td>1/8 dose</td>
<td>-----</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>Placebo</td>
<td></td>
<td>10</td>
</tr>
<tr>
<td>D2</td>
<td>¼ dose</td>
<td>-----</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>Placebo</td>
<td></td>
<td>10</td>
</tr>
<tr>
<td>D3</td>
<td>½ dose</td>
<td>-----</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>Placebo</td>
<td></td>
<td>10</td>
</tr>
<tr>
<td>D4</td>
<td>Highest safe dose 2.5 µg</td>
<td>-----</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>Placebo</td>
<td></td>
<td>10</td>
</tr>
<tr>
<td>D5</td>
<td>Highest safe dose 5 µg</td>
<td>-----</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>Placebo</td>
<td></td>
<td>10</td>
</tr>
<tr>
<td></td>
<td><strong>Total</strong></td>
<td></td>
<td><strong>200</strong></td>
</tr>
</tbody>
</table>

**Design may help determine dose sparing effect of dmLT**
Trial site options for OEV-123 in 2017 – early efficacy studies in Travelers

- U.S. military personal deploying for training or humanitarian missions
- Finnish tourist to Benin
- Belgium military training in Gabon
ACE527: Multivalent, live attenuated vaccine candidate

- Clinically advanced candidate addressing both CFs and toxin (only 3 strains); estimate coverage at 75-85%.
- Safe, immunogenic, and protective when given with dmLT (protect seen at 6-7 mth).
- NVSI in China is conducting process development studies to determine if co-formulated vaccine is practical.
ACE527 + dmLT: Efficacy of 3-dose regimen against severe diarrhea

<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>

- Deeper dive into the immunological responses induced by the ACE 527 + dmLT are ongoing in an effort to better understand the basis for the greater protection seen in the adjuvanted vaccine group.
- PATH will sponsor a workshop in 3Q 2016 to review these data.
Early observations from more in-depth analysis of immune response induced by ACE527 vaccine + dmLT: Immunoproteomics

<table>
<thead>
<tr>
<th></th>
<th>Placebo (n=10)</th>
<th>ACE527 (n=12)</th>
<th>ACE527+dmLT (n=13)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frequency Counts</td>
<td>0 4 10</td>
<td>1 12 13</td>
<td>0 4 5</td>
</tr>
<tr>
<td></td>
<td>0 2 0</td>
<td>0 0 0</td>
<td>0 1 0</td>
</tr>
<tr>
<td></td>
<td>3 1 0</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1 1 0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Positive is ≥2-fold increase from Day 0 to Day 7

Other antigen enhanced in dmLT group: FliC and Ag 43

PCF071 is related to the CS1 colonization expressed by ACE527

Data courtesy of ADi, Inc. and PATH microarray consortium
Vectored ETEC vaccines under development

- **ShigETEC**: Live attenuated, LPS-free, *Shigella* vaccine expressing LT-ST fusion protein (EVELIQURE); poised to begin Phase 1 in 2017.

- **CVD hybrid *Shigella*-ETEC vaccine strategy**: Development consortium being formed to move back into the clinic (UMB).

- **TyphETEC**: ZH9 typhoid vectoring LT/ST toxoid and CF/CS antigens (PROKARIUM); expansion to include *Shigella* antigens also being considered, but will remain in preclinical development for near future.
ETEC Vaccine Development Landscape: Subunit Candidates
ETEC fimbrial tip adhesin (FTA) vaccine

- Vaccine concept - Development of antibodies to adhesive fimbrial subunits will abrogate colonization step and help prevent subsequent disease. Disease can be further minimized by also eliciting antibody to major fimbrial subunits (pilin).
- US Naval Medical Research Center (NMRC) developed recombinant donor strand complemented FTA proteins expressing the pilin-adhesin or two major pilin subunits as potential vaccine antigens.
- A multivalent vaccine approach covering four of the most prevalent colonization factors plus and LT component is the current approach; estimated to give ≥80% of most common strains.

<table>
<thead>
<tr>
<th>Antigen</th>
<th>CfaEB</th>
<th>CotDA</th>
<th>CsbDA-CooA</th>
<th>CssBA</th>
</tr>
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<tbody>
<tr>
<td>Structure</td>
<td><img src="image1.png" alt="Structure" /></td>
<td><img src="image2.png" alt="Structure" /></td>
<td><img src="image3.png" alt="Structure" /></td>
<td><img src="image4.png" alt="Structure" /></td>
</tr>
<tr>
<td>Estimated coverage</td>
<td>Class 5a: CFA/I, CS14, CS4</td>
<td>Class 5C: CS2</td>
<td>Class 5b: CS1, PCF071, CS17, CS19</td>
<td>CS6</td>
</tr>
</tbody>
</table>
FTA: Proof of principle of adhesin approach

Volunteer anti-CfaE bovine IgG passive immunoprophylaxis trial

- Hyperimmune anti-CfaE bovine colostral preparation.
- Passive immunoprophylaxis trial in volunteers.
- Passive protective efficacy observed against:

<table>
<thead>
<tr>
<th>End point</th>
<th>PE (%)</th>
<th>95% CL</th>
</tr>
</thead>
<tbody>
<tr>
<td>All diarrhea</td>
<td>63</td>
<td>2, 86</td>
</tr>
<tr>
<td>Moderate-severe diarrhea</td>
<td>84</td>
<td>-6, 98</td>
</tr>
</tbody>
</table>

Data courtesy of NMRC

Protective efficacy of Class 5 adhesin-pilin fusions in *Aotus nancymaiae* model of ETEC diarrhea

- Several vaccination-challenge studies performed in NHP model of ETEC diarrhea.
- Vaccines were tested independently and compared to PBS control vaccinations.

<table>
<thead>
<tr>
<th>Class</th>
<th>Vaccine</th>
<th>Route</th>
<th>PE(%)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>5a</td>
<td>CfaE, LTB</td>
<td>ID</td>
<td>84</td>
<td>0.015</td>
</tr>
<tr>
<td>5b</td>
<td>CsbDA-CooA, LTB</td>
<td>ID</td>
<td>70</td>
<td>0.05</td>
</tr>
<tr>
<td>5c</td>
<td>CotDA, LTB</td>
<td>ID</td>
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<td>0.07</td>
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</tbody>
</table>

1Protective efficacy against CF-homologous ETEC Strain
2Fisher's exact test, one-tailed
ETEC FTA vaccine GHVCI collaboration

- Based on favorable Phase 2b challenge study and NHP protection data, IDRI and Sanofi Pasteur established the Global Health Vaccine Center of Innovation (GHVCI), funded in part from the Bill & Melinda Gates Foundation (October 2015).
- GHVCI/IDRI is a key partner in ETEC FTA vaccine development with Sanofi Pasteur, PATH, and NMRC.
  - Leverages broad vaccine expertise and should accelerate development by efficient application of resource-sparing methods.

<table>
<thead>
<tr>
<th>CY16</th>
<th>CY17</th>
<th>CY18</th>
<th>CY19</th>
<th>CY20</th>
<th>CY21</th>
<th>CY22</th>
<th>CY23</th>
<th>CY24</th>
<th>CY25</th>
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**Manufacturing and Preclinical Studies** (Mouse, *Aotus nancymae*, *Rhesus macaques*) - Multivalent Formulation for IM Administration

- Protein process development and cGMP manufacturing*
- Small animal and NHP studies formulation, dosing schedule
- GLP Toxicology

**Clinical Studies - Multivalent Formulation - Pediatric and Travelers/Military Indications**

- **Pediatric Indication**
  - CssBA+dmLT GLP toxicity
  - CssBA+dmLT ID v. IM Phase 1

- **Travelers/Military Indication**
  - CssBA+dmLT Phase 2b

- **Multivalent Formulation**
  - Phase 1 – FIH
    * Dose escalation
    * +/- adjuvant
  - Phase 1 - age de-escalation
  - Phase 2 Dose-ranging and non-interference (2 studies)
  - Phase 2b ST+
    - only challenge strain
  - Phase 2 expanded safety / field site development
  - Phase 3 - Travelers/Military

- **WHO Pre-Qualification**
- **FDA BLA**

*Includes analytical assay development, qualification and validation and ongoing stability testing
### ST toxoid development

**Challenge:** Lack of immunogenicity  
**Solution:** Couple to carrier  
**Approach:** Chemical conjugation, Genetic fusion

<table>
<thead>
<tr>
<th>Toxicity</th>
<th>Cross-reactivity (uro)guanylin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mutation(s)</td>
<td>Selective exposure of epitopes</td>
</tr>
<tr>
<td>Saturation mutagenesis</td>
<td>Epitope mapping</td>
</tr>
</tbody>
</table>

#### Status
- N12, A14 and L9 primary targets for attenuation.  
- Being developed as conjugate or fusion.  
- Both constructs induced toxin-neutralizing antibodies in mice and pigs.  
- Vector expression under development.  
- Clinical entry likely in at least 2-3 years.

**Rational design of immunogen**
- Detoxifying mutations  
- Optimized exposure of neutralizing epitopes  
- Disruption of cross-reactivity

Summary courtesy of A. Taxt of EntVac consortium and University of Bergen
Multiepitope Fusion Antigen (MEFA) vaccine for ETEC

**Concept of MEFA**
- Methods developed by Weiping Zhang (Kansas State University) in collaboration with David Sack (Johns Hopkins).
- One fusion protein can express the dominant epitopes of key ETEC antigens.
- Avoids need for multiple strains to express multiple CFA and toxin antigens.
- Could be given either orally or by injection.
- Injected MEFA is:
  - Immunogenic in mice and pigs.
  - Protective in piglet model.
  - Being evaluated in rabbits against colonization.

**Status of development**
- CFA-MEFA protein was developed with 7 or more CF antigens.
- Toxoid MEFA was developed with both LT and ST (ST has not been exploited as a vaccine component before).
- The two MEFA proteins could be co-administered to induce both anti-CFA and antitoxin immunity.
- A single CFA-Toxoid MEFA also developed – includes both CFA and toxoid epitopes in a single fusion.
- Could be used as carrier protein for *Shigella* conjugate vaccine.

Summary courtesy of W Zhang and D Sack of KSU and JHBSHPH
Additional ETEC proteins may have vaccine potential

**Plasmid-based: ETEC-specific**

- **EtpA** – accessory adhesin on flagella; appears to also help in LT delivery.
- **EatA** – serine protease mucin components and helps deliver LT toxin; 80% homology with SepA of *S. flexneri*.

Recent study found more than half of ETEC strains examined made EtpA and/or EatA both contribute to anti-colonization immunity in the mouse model (Luo, Q et al., 2014 PloS one).

In mouse studies, an inverse relationship has been shown between anti-EtpA IgA and IgG fecal antibody levels and reduced colonization by ETEC (Fleckenstein et al, CVI 2016, In press).

**Chromosomal-based: May be present in commensals**

- **Yghj** – metaloprotease with mucinase activity.
- **EaeH** – adhesin that acts in concert with CF/CSs and with EtpA and EatA to help deliver LT.
- **Ag 43** – surface exposed autotransporter; contributes to anti-colonization intestinal immunity in mouse model; recognized by sera from experimental and natural infection.

Summary and discussion
## Current status of ETEC vaccine candidates

<table>
<thead>
<tr>
<th>Clinical Status</th>
<th>Candidates</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Active (in clinical trials)</td>
<td>ETVAX, FTA</td>
<td>2</td>
</tr>
<tr>
<td>Inactive (passed phase 1, no further work scheduled)</td>
<td>ACE527, TyphETEC</td>
<td>2</td>
</tr>
<tr>
<td>Pending (Phase 1 trial anticipated)</td>
<td>ShigETEC, GuaBA-ETEC</td>
<td>2</td>
</tr>
<tr>
<td>Preclinical (Clinical feasibility still to be determined)</td>
<td>LT/ST toxoids, MEFA, Novel conserved antigens (EtpA, EatA)</td>
<td>3</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td><strong>9</strong></td>
</tr>
</tbody>
</table>
Assessment of WHO role for ETEC 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
Summary: ETEC and Shigella burden

- Mortality and morbidity estimates for both agents remain in flux – new IHME updates projected for late June with follow-on BMGF workshop 20-21 July 2016.

- Potential disease burden of ETEC and *Shigella* goes beyond direct mortality and morbidity after infection – stunting and chronic diseases are related to high burden impact on quality of life and economic potential.

- Further studies are needed to capture disease burden and disease heterogeneity both between and within countries in endemic areas (hyperendemic areas, focal hot spots).

- A combined ETEC-*Shigella* vaccine is projected to have the most significant impact on case burden in children under 5 living in endemic areas (~37% of burden averted in U5’s projected for 2040 even with other preventive interventions scaled-up, including rotavirus vaccine).
Summary: ETEC vaccine landscape

Overview

- Promising cellular and subunit vaccine candidates are moving forward in clinical development.
- DmLT has been shown to benefit elements of humoral and cellular immune responses to ETEC vaccine candidates (ETVAX, ACE527, and FTA).

ETVAX

- In-depth studies indicate memory to ETVAX lasts for at least 1-2 years and breadth of mucosal antibody response following primary immunization is broader than anticipated, suggesting potential for broader strain coverage.
- ETVAX currently in descending-age study in Bangladesh and has moved into 1-2 yr. olds; dmLT safely given to toddlers (2-5 yrs) for first time. Evidence of dmLT dose sparing pending.
- Study to evaluate ETVAX administered in EPI scheduled to begin in late 2016; initial Phase 2b efficacy studies in travelers planned for early 2017.
- Meeting with WHO held to review prequalification requirements for ETVAX; EU approached about licensing strategy.

ACE527

- DmLT may help improve protection by broadening immune response to include other ETEC antigens present in vaccine (i.e. EatA)
Summary: ETEC vaccine landscape (continued)

FTA
• Formation of new ETEC FTA Vaccine GHVCI Collaboration should accelerate development and testing of complete adhesion-based subunit vaccine; testing of complete vaccine projected for 2019.
• Studies to evaluate CS6 as a protective vaccine antigen targeted to begin in 2017.

ST toxoid
• Projected to go into first clinical trial in 2017 (vectored in ShigETEC).
• Impressive advances have been made in attenuating ST by targeted AA substitution in key position(s) in peptide; could be used in both cellular and subunit vaccine candidates.

MEFA technology
• Shows promise in early porcine studies; fusions to cover for human ETEC strain know being constructed for testing in rabbits.
• Has flexibility to be vectored or given as subunit, also expandable to provide coverage for most major CF/CS plus more conserved antigens..

Other
• Omics technologies have identified new conserved antigen (EtpA and EatA) that may help improve both cellular and subunit vaccine approaches by improving candidate ability to block LT toxin delivery and intestinal colonization.
Discussion