Human challenge study with a *Shigella* bioconjugate vaccine: Analyses of clinical efficacy and correlate of protection.

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Summary

Background: Shigellosis is a major cause of moderate to severe diarrhea and dysentery in children under 5 years of age in low and middle-income countries. The Flexyn2a vaccine conjugates the O-polysaccharide of *Shigella flexneri* 2a to *Pseudomonas aeruginosa* exotoxin A. We describe a Phase 2b proof-of-concept challenge study that evaluated safety, immunogenicity, and efficacy of the Flexyn2a vaccine to protect against shigellosis.

Methods: In this randomized, double blind, placebo-controlled trial, healthy adults were randomized 1:1 to receive Flexyn2a (10µg) or placebo intramuscularly, twice, 4 weeks apart, followed by challenge with 1500 colony forming units (CFUs) of *S. flexneri* 2a strain 2457T. The primary outcome was vaccine-induced protection. *S. flexneri* 2a lipopolysaccharide (LPS)-specific immune responses were assessed.

Findings: Sixty-seven subjects were enrolled, 34 received vaccine and 33 placebo. The vaccine was well tolerated; the majority of adverse events were mild in nature. Thirty vaccinees and 29 placebo recipients received the *S. flexneri* 2a challenge. Vaccination resulted in a 30.2% reduction in shigellosis compared with placebo (p=0.11). Vaccine efficacy was more robust against severe disease, reaching 51.7% (p = 0.015) against moderate/severe diarrhea or dysentery concurrent with fever or severe enteric symptoms and 72.4% (p=0.07) against more severe diarrhea (≥10 lose stools or ≥1000 g loose stools/24 hours). Vaccinated subjects were less likely to need early antibiotic
intervention following challenge (PE=51.7%; p=0.01). In those who developed shigellosis, vaccinated subjects had a lower disease severity score (p=0.002) than placebo-recipients. Additionally, LPS-specific serum IgG responses in Flexyn2a recipients was associated with protection against disease (p=0.0016) and with a decreased shigellosis disease score (p=0.002).

**Interpretation:** The Flexyn2a bioconjugate vaccine was immunogenic, well tolerated and protected against severe illness after *Shigella* challenge and is a promising *Shigella* vaccine construct. We identified a strong association between anti-*S. flexneri* 2a serum IgG and a reduction in disease outcomes. (Clinicaltrials.gov, NCT02646371.)

**Funding:** Funding for this study was through a grant from the Wellcome Trust.

*Preliminary data presented at the Vaccines for Enteric Diseases meeting, 2017. Word count: 3084*
Research in Context

Evidence before this study. There is currently no licensed Shigella vaccine. A pubmed search for “shigella” AND “vaccine” unlimited by language, restricted to clinical trials revealed 70 articles about several vaccine constructs, including the bioconjugate vaccine described in this manuscript. No other bioconjugate vaccines are in clinical trials, although there are several other Shigella vaccines, including a chemical conjugate vaccine in development. An earlier generation of conjugate vaccine, developed by John Robbins and colleagues was found to be safe and immunogenic and effective in Israeli military adults, but not in in children between 1-4 years of age. A phase 1 trial of the Flexyn2a vaccine has been published, describing the initial safety and immunogenicity of this vaccine. This Phase 2 study was designed to assess the efficacy of the Flexyn2a vaccine against challenge with S. flexneri 2a in a controlled human infection study.

Added value of this study. This is the first study investigating the efficacy of the Flexyn2a Shigella bioconjugate vaccine in adults. This study utilized the controlled human challenge model to demonstrate the efficacy of the Flexyn2a vaccine against clinical shigellosis, and the vaccine was found to be particularly effective against severe shigellosis. This study also confirmed the safety and immunogenicity observed in the earlier Phase 1 trial. It is also the first study to evaluate efficacy utilizing the recently published consensus endpoints for Shigella controlled human challenge studies.
Implications of all the available evidence. This study shows that the Flexyn2a vaccine is well tolerated, immunogenic, and is protective against severe shigellosis, although protection against the per-protocol definition of shigellosis did not reach statistical significance. The results also help to identify a potential correlate of immunity for Shigella, the LPS-specific serum IgG response and also suggests that a Shigella vaccine impacting on the incidence and severity of Shigellosis can potentially reduce the need for antibiotic treatment. This study is an important step forward toward the eventual licensure of a vaccine against Shigella.
Introduction

*Shigella* spp. cause moderate to severe diarrhea and dysentery predominantly in children under 5 years of age in low to middle income countries\(^1\). Shigellosis is often characterized by systemic and enteric symptoms and can be life threatening in vulnerable hosts\(^1\)-\(^3\). In addition to the mortality, morbidity and long-term consequences associated with shigellosis (i.e. stunting and wasting)\(^4\)-\(^7\), reports of spreading resistance to antibiotics highlight the need for primary prevention\(^8\).

Despite ongoing Shigella vaccine development efforts for almost 100 years\(^9\),\(^10\), no licensed vaccine is available. Previously, chemical conjugates were shown to be effective in adults but not in children under 3 years of age\(^11\),\(^12\) and protection was associated with the LPS-specific serum IgG response post-vaccination. These early products were never commercially developed. A vaccine that is simple to manufacture and more effective in children is greatly needed, and conjugate vaccines produced with bioconjugation technology have shown great potential\(^13\)-\(^16\). Flexyn2a is a bioconjugate vaccine composed of the O-polysaccharide of *Shigella flexneri* 2a enzymatically linked to the exotoxin A of *Pseudomonas aeruginosa* (EPA) using a reproducible and greatly simplified conjugation process\(^17\). In a recent Phase 1 study, Flexyn2a exhibited a good safety and robust immunogenicity profile\(^15\). We describe here a proof-of-concept efficacy study performed to demonstrate the ability of Flexyn2a to protect against shigellosis following challenge with *S. flexneri* 2a strain 2457T and to determine if LPS-specific serum IgG correlates with a reduction in disease outcomes. This vaccination strategy has been broadened to create a multivalent *Shigella* vaccine
targeting the most
relevant circulating strains of *Shigella* in low- and middle-income countries and a phase 1/2 clinical trial in east Africa is currently ongoing (NCT04056117).

**Methods**

**Clinical Trial Design:** The trial was randomized, double-blinded and placebo-controlled. Two cohorts of 36 healthy adult volunteers were planned to be successively enrolled. In each cohort, a vaccination phase was followed by a challenge phase, with up to 30 volunteers from each cohort to be challenged.

**Study Oversight:** The study was conducted at the Johns Hopkins Bloomberg School of Public Health Center for Immunization Research (CIR). The challenge phase was conducted at the CIR inpatient unit at the Johns Hopkins Bayview Medical Campus. All subjects provided written informed consent. The trial was approved by the Western Institutional Review Board in compliance with all federal regulations governing the protection of human volunteers. LimmaTech served as the sponsor of the study, and developed the study design with the investigators. The investigators were responsible for study conduct and management. (Clinicaltrials.gov registration NCT02646371).

**Vaccine:** The Flexyn2a vaccine is produced *in vivo* in *E. coli* and subsequently purified as previously described\(^{15}\). Each dose contains 10μg of *Shigella flexneri* 2a O-polysaccharide and approximately 50μg EPA. The product has been characterized extensively including assays for content, purity, and structure\(^{17}\). The vaccine (or saline placebo) was administered twice, one month apart, via intramuscular injection with a dose volume of 0.5mL.
**Challenge Strain:** The 2457T *Shigella flexneri* 2a challenge strain is a well-characterized *Shigella* strain manufactured under current Good Manufacturing Practice conditions at the Walter Reed Army Institute of Research Pilot BioProduction Facility in Silver Spring, Maryland. The target challenge-dose of 1500 CFU was chosen based on published studies as one that could be safely utilized and would yield a sufficiently high shigellosis attack rate (AR). The predetermined acceptable range for the challenge inoculum was 1500-2000 CFU.

**Study Population and Enrollment Criteria:** A consensus description of the methods involved in the conduct of a *Shigella* challenge have recently been published. Essentially, volunteers were healthy male and nonpregnant female adults between 18-50 years of age recruited from the Mid-Atlantic area. Informed consent was a rigorous and iterative process to ensure comprehension of the trial and their participation. To ensure eligibility criteria were met, medical history, laboratory tests and a complete physical exam were performed. Among other requirements, volunteers were eligible if they had no significant medical history or exam findings of inflammatory arthritis, chronic gastrointestinal problems or irritable bowel syndrome, were HLA-B27 negative (to decrease the risk of reactive arthritis after challenge), and had no recent history of traveler’s diarrhea, or participation in other *Shigella* trials within 3 years. In addition, eligibility was limited to volunteers with a *S. flexneri* 2a-LPS specific serum IgG ELISA endpoint below 2500 in an attempt to recruit a *Shigella*-naïve population.

**Randomization and Masking:** This study was a randomized, double blind, and placebo controlled. For each cohort, randomization was done in six blocks
(randomly}
ordered) of six subjects each, via the interactive web response system (IWRS) AdvantageEDC by the CRO, the EMMES Corporation, and the treatment key was provided to the pharmacy which dispensed the vaccine. The volunteers, site personnel, laboratory staff and monitors were blinded, as were some representatives of the Sponsor. The Investigational pharmacist, other personnel at the CRO and at the Sponsor were unblinded, but had no access to the volunteers or study data except for the pharmacy and laboratory records. The blinding of the subjects was maintained throughout the entire study.

**Safety Monitoring and Study Procedures:** Many of the study procedures for a *Shigella* human challenge study have since been published\(^{21-23}\). During the vaccination phase, volunteers were followed as outpatients for safety and completed a surveillance document for 7 days post each vaccination. Solicited and unsolicited adverse events were collected for 28 days after the last vaccination. At the end of the vaccination phase, volunteers were admitted to the inpatient unit one day prior to challenge. On the day of challenge, subjects consumed 120mL of bicarbonate buffer, and immediately after, the freshly prepared challenge inoculum of *S. flexneri* 2a strain 2457T in 30mL of bicarbonate buffer. Physical assessments were performed daily; vital signs were measured thrice daily. From the day of the challenge and until discharge, all stools passed on the unit were assessed for consistency, weight, and gross blood\(^{20}\). Daily stool cultures were performed for qualitative and quantitative measures of the challenge microorganism. Selected colonies were serotyped using commercial agglutination serum (Denka).
Subjects with loose stools were provided oral rehydration and closely monitored for signs and symptoms of hypovolemia and were treated with intravenous fluids as necessary. On or before (if indicated) day 5 post-challenge, subjects were treated with an antibiotic (ciprofloxacin or trimethoprim/sulfamethoxazole) twice daily for 3 days.

Subjects were eligible for discharge once they had at least 2 Shigella-negative stool cultures and had received at least 2 doses of antibiotics. Volunteers had an outpatient visit one month post-challenge, and a safety phone call for serious adverse events and adverse events of special interest at 6 months post-challenge. Challenge-related solicited and unsolicited adverse events were collected for 1 month from the day of challenge.

**Definitions:** The primary clinical endpoint was shigellosis, defined as severe diarrhea OR moderate diarrhea with fever or with one or more moderate constitutional or enteric symptom OR dysentery. The definitions of diarrhea are located in the Supplement. More severe shigellosis was defined in a post-hoc analysis as at least moderate diarrhea or dysentery, with fever or severe enteric symptoms. A slight modification of this post-hoc definition was subsequently endorsed for use in Shigella CHIMs studies by a convening of experts, held in 2017.

**Endpoints:** The shigellosis AR after challenge was the primary endpoint. Among others, secondary efficacy endpoints included weight and number of loose stools and incidence of fever and enteric symptoms. In addition, safety and immunogenicity endpoints were assessed (see supplement). The Shigellosis
Disease Score described by
Porter et al\(^4\) was used to assess whether the severity of disease experienced by the vaccinees and placebo recipients were comparable. An independent adjudication committee determined the endpoints of each subject.

**Immunogenicity Assessments:** Venous whole blood was collected prior to each vaccination, 7- and 28-days post-vaccination as well as before and 3, 7 and 28 days after challenge. Serum was separated from whole blood and frozen until assayed by ELISA. *S. flexneri* 2a LPS-specific serum IgG antibody titers were determined as previously described\(^{15,25}\). A serological responder was defined *apriori* as a ≥ 4-fold increase in titer over baseline.

**Statistical Analysis:** Assuming an attack rate for diarrhea in the placebo group estimated to be 70% and an attack rate (AR) of no higher than 30% in the vaccine group (equivalent to >57% protective efficacy), a total of 28 to 30 subjects per group was chosen to allow for at least 80% power to detect a significant difference (p<0.05; lower bound of 95% confidence interval around point estimate of efficacy of > zero) in attack rates between the vaccine and placebo groups. The shigellosis AR was presented as the proportion of subjects per group with shigellosis after challenge. Vaccine efficacy was calculated (VE = (AR\(_{\text{placebo}}\) – AR\(_{\text{vaccinees}}\))/(AR\(_{\text{placebo}}\))*100%) along with exact unconditional 95% confidence intervals (CI). Following the hypothesis that the vaccine reduces the attack rate, at this early stage of the program the effect in only one direction (protective effect) was considered. AR were compared with the unconditional exact 1-sided Barnard test. Analyses were 1-tailed and statistical significance attributed to p≤0.05.

Statistical and data analyses were conducted jointly between investigators from each institution.
The Shigellosis Disease Score was calculated as described previously \(^\text{24}\) and was analyzed with a 2-sided alpha. Differences in symptoms severity were assessed based on the exact test of equality of row means using modified-ridit scores.

Antibody titers were summarized as geometric mean titers (GMT) along with 95% confidence intervals. The ratios of GMT and corresponding 95% CI’s between treatment groups were calculated and groups were compared by Student’s t-test of log\(_{10}\)-transformed values. The percentage of subjects reaching a four-fold increase in serum IgG titers compared to baseline was calculated for each group and compared between-groups with Fisher’s exact test. Spearman correlation analyses were performed between log\(_{10}\)-transformed antibody titers and Shigella Disease Score, symptoms, maximum number or weight of loose stool.

**Role of the funding source:** The funder of the study (The Wellcome Trust) had no role in the study design data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

**Results**

**Demographics:** One hundred ninety-six potential volunteers were screened, and 67 volunteers were enrolled (Figures 1 and 2, Table 1). The most common reason for exclusion was a *S. flexneri* 2a LPS-specific serum IgG titer \(\geq 2500\) at screening (64 volunteers). Following randomization, 34 volunteers received Flexyn2a and 33 received
placebo. Out of the planned 60 subjects, 59 were challenged: 30 vaccinees and 29 placebo recipients (Figure 1, Figure 2). Thirty-one percent of the volunteers were women and 80.6% were Black or African-American. The median age of participants was 34.8 years (Table 1). The first Cohort received their first vaccination on December 15, 2015, and the second cohort their first vaccination on March 23, 2016.

**Safety:** The Flexyn2a vaccine was well-tolerated and the safety data collected generally confirmed the Phase 1 study observations. The most commonly reported adverse events were headache 14.7% (95% CI: 5% – 31.1%) in vaccinees and 30.3% (95% CI: 15.6% – 48.7%) in placebo recipients and pain at the injection site 26.5% (95% CI: 12.9% – 44.4%) in vaccinees and 18.2% (95% CI: 7% - 35.5%) in placebo recipients (Table 2). The majority (75.9%) of the adverse events were of mild intensity. No serious adverse events (SAEs) occurred during this study, and no subjects discontinued participation due to adverse events.

**Efficacy Data:** The *S. flexneri* 2a strain 2457T challenge dose administered to the first and the second cohort of volunteers was 1510 and 1707 CFUs, respectively. The shigellosis AR in placebo recipients in cohorts 1 and 2 was similar (60.0% and 64.3%, respectively), with a cumulative AR of 62.1%.

VE was 30.2% against the primary definition of shigellosis (Table 3) (p=0.11) and increased to 51.7% against more severe shigellosis (p=0.02). In addition, Flexyn2a protected vaccinees against more severe diarrhea (VE=72%; p=0.07), and reduced the need for early antibiotic treatment (VE=51.7%; p=0.01) and intravenous fluid administration (VE=47.9%; p=0.05) (Table 3).
weight of loose stools in any 24-hour period (geometric mean (GM) 274 gm. vs. 528 gm., p=0.009) as well as lower maximum number of loose stools in any 24-hour period (mean 4.8 vs. 7.1, p= 0.022) (Figure 3). The incidence of fever or any enteric symptoms was significantly lower in the Flexyn2a group compared with placebo (for any symptom of at least moderate p=0.044, or severe or greater intensity p=0.011). The severity of key symptoms such as fever, vomiting, abdominal cramps and myalgia (but not nausea, arthralgia, rigors, tenesmus or fecal urgency), was significantly lower among vaccinees (Figure 4). There was a significant difference (p=0.02) in the Shigellosis Disease Score$^{24}$ between the vaccinees (median: 1.6; IQR: 0-4.3) and placebo recipients (median: 4.2; IQR: 0.5-6.0), even if they met the primary endpoint (Figure 5A). In addition, vaccinees that met the primary definition of shigellosis had lower disease severity scores than placebo recipients (median score 5, IQR 3.5-5 vs. median score 6, IQR 5.5-7; p=0.002 Figure 5B). No difference in disease severity score was observed between groups of vaccinees and placebo recipients that did not show symptoms of shigellosis (Figure 5C).

**Challenge strain shedding:** Forty-three volunteers shed the *S. flexneri* 2a 2457T challenge strain, 22 (73%) placebo recipients and 21 (72%) Flexyn2a recipients. The CFUs detected in the stools of shedding subjects was similar in both groups, with a mean value of 6.20 (95% CI 5.68 to 6.72) and 5.79 (5.27 to 6.31) log$_{10}$CFU/g of stool in Flexyn2a and placebo, respectively (Table 4). The 13 vaccinees and the 18 placebo recipients adjudicated as having shigellosis all shed the challenge organism with similar levels (about 7 and 6 log$_{10}$ *Shigella* CFU/g of stool, respectively) except for 1 vaccinee with no shedding detected.
**Serological Responses:** A 4-fold or greater rise in serum IgG titers directed to *S. flexneri* 2a LPS was seen in 76.5% of vaccinees after the first dose, which increased to 81.8% after the second vaccination (Figure 6, Table 5). Despite randomization, the baseline serum IgG titers directed to *S. flexneri* 2a LPS were slightly higher (1.5-fold, p= 0.04) in the Flexyn2a recipients than in the placebo recipients (Figure 6, Table 5). *S. flexneri* 2a LPS-specific serum IgG responses increased from a baseline GMT of 2,172 (95% CI 1722-2740) to 23,119 (95% CI 12,704-42,073) on day 28 after the first vaccination with Flexyn2a (Figure 6, Table 5). Neither the second vaccination (GMT 19,896 on day 55; 95% CI 11,951-33,124), nor the subsequent challenge with *S. flexneri* 2a (GMT 18,958 on day 84; 95% CI 11,164-32,192) increased the LPS-specific serum IgG titer. Placebo recipients had baseline levels of *S. flexneri* 2a LPS-specific serum IgG prior to challenge with a GMT of 1,459 (95% CI 1,079-1,973). The LPS-specific serum IgG increased to 3,805 (2,609-5,551) one month after challenge. The level of *S. flexneri* 2a LPS-specific serum IgG elicited following vaccination was 5.0-fold higher than placebo recipients following challenge (p< 0.0001). A similar trend was observed for *S. flexneri* 2a LPS-specific serum IgA responses following vaccination, confirming the Phase 1 study results and data are included in a companion manuscript which focuses on the immunological results.

**Correlate of Protection Analyses:** In the Flexyn2a group, vaccinees protected from shigellosis had 5.1-fold higher *S. flexneri* 2a -LPS-specific serum IgG GMTs at time of challenge compared to vaccinees developing shigellosis (p=0.002) (Figure 7A).
Consistent with these findings, subjects with increasing LPS-specific serum IgG titers at the time of challenge was inversely correlated with the Shigellosis Disease Score (Spearman R=-0.55, p=0.002) (Figure 7B), loose stool weight (Spearman R=-0.44, p=0.016) and number of loose stools (Spearman R=-0.46, p=0.01) (Figure 7C).

Discussion

The results of our trial indicate that the bioconjugate Flexyn2a candidate vaccine is safe, induces a robust serologic response to S. flexneri 2a LPS and provides partial protection against shigellosis in a controlled human infection model. In this setting, Flexyn2a is more efficacious against severe shigellosis. Following a recent publication by McLennan et al\textsuperscript{23}, VE was also calculated using the published “shigellosis-consensus-definition in challenge trials”. This definition included moderate or severe diarrhea or dysentery with fever or a moderate or severe enteric symptom. Using this definition in a post-hoc analysis, the VE was 37.5%. This definition was not available at the time of this study design, and in fact, this study was used in the development of the consensus definition.

In addition, to the Flexyn2a vaccine being more effective at preventing severe shigellosis, placebo recipients had higher diarrheal stool outputs, more severe and frequent clinical symptoms including fever, were treated earlier with antibiotics and tended to have greater needs for intravenous fluids for dehydration than did the Flexyn2a group. The Flexyn2a recipients also had a lower overall shigellosis disease
severity score than the placebo recipients, even if they developed shigellosis, indicating that the breakthrough cases in vaccinees were milder.

As previously demonstrated with other conjugate vaccines, parenteral vaccination can be efficacious against invasive disease and has the potential to protect against mucosal pathogens\textsuperscript{26,27}. Also, in this human challenge model, the degree of vaccine efficacy is greater in more severe outcomes compared to milder infections; the latter may require a more robust immune response. When considering a vaccine for travelers, or a vaccine to prevent hospitalization of children, it is far more important to prevent severe disease than mild disease, as the more severe the disease, the greater the morbidity and mortality. Prevention of more severe disease in both travelers and children will also likely lead, as shown in this study, to a reduced need for antibiotic intervention, which would add greater public health benefit to vaccine use.

Previous studies have demonstrated that challenge with \textit{Shigella} elicits an immune response that confers protection against a subsequent homologous challenge\textsuperscript{28-30}. These results highlight the potential of the LPS-specific serum IgG immune response in achieving protection and reducing severity of disease, which is consistent with previous reports of the relevance of LPS-specific serum IgG as a marker of protection against shigellosis\textsuperscript{31-34}. A number of other mucosal and systemic immunity measures correlated with LPS-specific serum IgG responses and protection in this study. Those results are presented in a companion manuscript.
This study presents proof of concept that the Flexyn2a bioconjugate vaccine is able to protect against severe shigellosis outcomes following challenge and elicit a robust LPS-specific serum IgG immune response which correlates with protection against shigellosis. For a broad impact on public health, the *Shigella* vaccine will have to protect against the most prevalent *Shigella* serotypes causing disease in young children in low to middle income countries. A quadrivalent bioconjugate is currently being tested in a phase 1/2 clinical trial in Kenya (NCT04056117).

**Acknowledgments**

We thank Wellcome Trust for having provided funding for this study and we thank the study volunteers without whom this trial would not have been possible. The study teams, both clinical and laboratory, worked incredibly hard during this study, and we are indebted to them. We are also grateful to the members of the adjudication committee and to the Emmes Corporation for data management and study monitoring.
Table 1. Demographics of enrolled volunteers.
N= number; %= percent; ITT= intention to treat; yrs= years
* These subjects reported mixed race

<table>
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<th>Vaccination Phase - Safety Population</th>
<th>Challenge Phase - ITT Population</th>
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<tr>
<td></td>
<td>Flexyn2a N=34 Placebo N=33 Total N=67</td>
<td>Flexyn2a N=30 Placebo N=29 Total N=59</td>
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<tr>
<td>Gender - N (%)</td>
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<tr>
<td>Female</td>
<td>10 (29.4) 11 (33.3) 21 (31.3)</td>
<td>9 (30.0) 8 (27.6) 17 (28.8)</td>
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<tr>
<td>Male</td>
<td>24 (70.6) 22 (66.7) 46 (68.7)</td>
<td>21 (70.0) 21 (72.4) 42 (71.2)</td>
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<td>Age (yrs)</td>
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<tr>
<td></td>
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<td></td>
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<td>22.3-50.3 22.3-50.3</td>
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<td>Race - N (%)</td>
<td>American Indian/Alaskan Native, Black or African American*</td>
<td></td>
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<tr>
<td></td>
<td>3 (8.8) 1 (3.0) 4 (6.0)</td>
<td>3 (10.0) 0 (0.0) 3 (5.1)</td>
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<tr>
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<td>Asian 0 (0.0) 1 (3.0) 1 (1.5)</td>
<td>0 (0.0) 1 (3.4) 1 (1.7)</td>
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<td>Black or African American 26 (76.5) 28 (84.8) 54 (80.6)</td>
<td>22 (73.3) 25 (86.2) 47 (79.7)</td>
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<td>Hispanic 1 (2.9) 0 (0.0) 1 (1.5)</td>
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<td>White 4 (11.8) 3 (9.1) 7 (10.4)</td>
<td>4 (13.3) 3 (10.3) 7 (11.9)</td>
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Table 2. Adverse events after vaccination.
AE= Adverse event; mod= moderate; %= percent

<table>
<thead>
<tr>
<th>Adverse event</th>
<th>Flexyn2a Number (%)</th>
<th>Placebo Number (%)</th>
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<tbody>
<tr>
<td></td>
<td>mild</td>
<td>mod</td>
</tr>
<tr>
<td>Any AEs</td>
<td>14 (41.2)</td>
<td>3 (8.8)</td>
</tr>
<tr>
<td>Pain/Tenderness at injection site</td>
<td>8 (23.5)</td>
<td>1 (2.9)</td>
</tr>
<tr>
<td>Erythema/redness at injection site</td>
<td>2 (5.9)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Induration/swelling at injection site</td>
<td>2 (5.9)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Headache</td>
<td>4 (11.8)</td>
<td>1 (2.9)</td>
</tr>
<tr>
<td>Vomiting</td>
<td>1 (2.9)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Myalgia</td>
<td>2 (5.9)</td>
<td>1 (2.9)</td>
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<td>Arthralgia</td>
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<td>0 (0.0)</td>
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<td>Nausea</td>
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<td>0 (0.0)</td>
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<td>Abdominal pain</td>
<td>1 (2.9)</td>
<td>1 (2.9)</td>
</tr>
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<td>Chills</td>
<td>2 (5.9)</td>
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</tr>
<tr>
<td>Sweats</td>
<td>1 (2.9)</td>
<td>0 (0.0)</td>
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</table>
Table 3. Attack rates and vaccine efficacy.

Shigellosis: severe diarrhea OR moderate diarrhea with [fever (oral temperature ≥38°C) or with one or more moderate constitutional or enteric symptom] OR [dysentery].
More severe shigellosis: defined in a post-hoc analysis as at least moderate diarrhea or dysentery, with fever or severe enteric symptoms.
More severe diarrhea: ≥10 or ≥1000 g loose stools within 24 hours.
Severe diarrhea: ≥6 or >800 g loose stools within 24 hours.
Moderate diarrhea: 4 to 5 or 401-800 g loose stools within 24 hours.
Dysentery: at least 2 loose stools with gross blood (confirmed by hemoccult) within 24 hours and any reportable constitutional symptom.
Constitutional/Enteric Symptoms: nausea, vomiting, abdominal cramps/pain, myalgia, arthralgia, rigors, tenesmus and fecal urgency.

§ Exact unconditional 95% confidence interval for vaccine efficacy
*Unconditional exact 1-sided Bernard test; analyses were 1-tailed and statistical significance attributed to p≤0.05.
Abbreviations: N= Number, CI= Confidence interval

<table>
<thead>
<tr>
<th>Attack Rate N(%)</th>
<th>Vaccine Efficacy</th>
<th>p-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Flexyn2a N=30</td>
<td>Placebo N=29</td>
</tr>
<tr>
<td>Shigellosis (primary definition)</td>
<td>13 (43.3)</td>
<td>18 (62.1)</td>
</tr>
<tr>
<td>More Severe Shigellosis (post-hoc definition)</td>
<td>8 (27.6)</td>
<td>16 (53.3)</td>
</tr>
<tr>
<td>Shigellosis (post-hoc Consensus paper23 definition)</td>
<td>11 (36.7)</td>
<td>17 (58.6)</td>
</tr>
<tr>
<td>Secondary Endpoints</td>
<td></td>
<td></td>
</tr>
<tr>
<td>More Severe Diarrhea</td>
<td>2 (6.7)</td>
<td>7 (24.1)</td>
</tr>
<tr>
<td>Received Early Administration of Antibiotics</td>
<td>9 (30.0)</td>
<td>18 (62.1)</td>
</tr>
<tr>
<td>Received IV Fluids</td>
<td>7 (23.3)</td>
<td>13 (44.8)</td>
</tr>
<tr>
<td>Number of subjects with moderate-severe diarrhea</td>
<td>15 (50.0)</td>
<td>17 (58.6)</td>
</tr>
<tr>
<td>Number of subjects with diarrhea of any severity</td>
<td>17 (56.7)</td>
<td>21 (72.4)</td>
</tr>
</tbody>
</table>
Table 4. Challenge strain shedding.
N=number; %= percent; CFU= colony forming unit; SD= standard deviation

Table 5. Anti-LPS serum IgG titers (median, range, geometric mean (GM) and 95% confidence interval (95% CI) by vaccinee or placebo recipient status, and the ratio of the titer of the vaccinees/placebo recipients. The percent (%) responder by serum IgG (4-fold or greater increase in serum IgG) titer after vaccination. N=number.

| Study Day | Flexyn2a | | | | Placebo | | | | | Ratio GM Flexyn2a/Placebo | | |
|-----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|--------------------------|----------|
|           | N        | Median   | GM       | % Responder | N        | Median   | GM       | Ratio | P-Value |
|           | (Range)  | (95% CI) | (95% CI) |          | (Range)  | (95% CI) | (95% CI) |       |         |
| Vaccination Phase | | | | | | | | | | | |
| Day 0     | 34       | 3200     | 2172     | N/A      | 30       | 1600     | 1459     | 1.5   | 0.04    |
| Day 7     | 33       | 3200     | 23119    | 76.5%    | 30       | 1600     | 1493     | 2.1   | 0.0004  |
| Day 28    | 34       | 25600    | 21998    | 81.8%    | 30       | 1600     | 1493     | 14.8  | <.0001  |
| Day 35    | 32       | 25600    | 19896    | 81.8%    | 30       | 1600     | 1493     | 14.3  | <.0001  |
| Day 55 (prechallenge) | 33 | 25600    | 19896    | 81.8%    | 30       | 1600     | 1493     | 14.3  | <.0001  |
| Challenge Phase | | | | | | | | | | | |
| Day 59 (3 days postchallenge) | 30 | 25600    | 20794    | 81.8%    | 29       | 1600     | 1454     | 14.3  | <.0001  |
| Day 63 (7 days postchallenge) | 30 | 25600    | 21280    | 81.8%    | 28       | 1600     | 2049     | 10.4  | <.0001  |
| Day 84    | 30       | 25600    | 18958    | 81.8%    | 28       | 3200     | 3805     | 5.0   | <.0001  |
Manuscript Figures

Figure 1: Study Design and Enrollment. Volunteers received 2 doses of the Flexyn2a vaccine or placebo 28 days apart followed by challenge with \textit{S. flexneri} 2a 28 days after the second dose. Immunological assessments were done before and after vaccination and after challenge.

Figure 2: Consort Diagram Study enrollment and subject disposition. BMI = body mass index.
Figure 3: The mean of the cumulative weight (Panel A) and number (Panel B) of loose stools after challenge in the Flexyn2a recipients (yellow line) and the placebo recipients (gray line). The table reports the maximum weight and number of loose stools within any 24-hour period after challenge.

N= number; g= grams; hrs=hours; GM= Geometric mean; IQR= interquartile range; SD= standard deviation
Figure 4: Constitutional and enteric symptoms experienced by volunteers after Sfl2a challenge. P= placebo recipients (n=29); V= vaccine recipients (n=30). Color indicates severity. The p-value for each symptom is along the top and reflects the difference in severity utilizing the modified Ridit Score.
**Figure 5:** Shigellosis disease severity score in challenged volunteers by Vaccinee or placebo recipient (Panel A), only those that developed shigellosis by vaccinee or placebo recipient (Panel B) and those that didn't develop shigellosis by vaccinee or placebo recipient status (Panel C).

**Figure 6:** Shows the anti-\textit{S. flexneri} 2a LPS serum IgG antibody titers by study day. Vac= Vaccination. C-1 = 1 day prior to challenge. C7 = 7 days post-challenge.
Figure 7: Panel A shows the prechallenge serum IgG to *S. flexneri* 2a LPS in the vaccine and placebo recipients broken down by whether they met the primary objective of Shigellosis. Panel B, the relationship between pre-challenge anti-LPS serum IgG to Shigella disease severity score is demonstrated for the recipients of the Flexyn2a vaccine. Panel C demonstrates the relationship of the pre-challenge anti-LPS serum IgG to maximum stool weight (blue circles) and number (red triangles) in 24 hours.
References


26. Pinto LA, Kemp TJ, Torres BN, et al. Quadrivalent Human Papillomavirus (HPV) Vaccine Induces HPV-Specific Antibodies in the Oral Cavity: Results From the Mid-Adult


Author contributions:

KRT: helped with study design, oversaw conduct of study and care of volunteers, assisted with data analysis and interpretation, drafted and edited manuscript.

CA: Helped with study design, data analysis, interpretation, did significant writing and editing of manuscript.

PM: Helped with data interpretation, editing of paper

ALB: helped with study design, helped prepare challenge strain, oversaw laboratory assays and analysis, contributed to manuscript and editing.

AMD: helped with statistical and laboratory design and analysis, contributed to manuscript and editing.

RWK: helped with study design, provided challenge strain, conducted laboratory assays and analysis, contributed to manuscript and editing.

CKP: Helped with study design, developed statistical plan, contributed to data analysis and statistical analysis, did significant writing and editing of manuscript.

SC: oversaw laboratory assays and analysis, contributed to manuscript and editing.

KAC: conducted laboratory assays and analysis, contributed to manuscript and editing. JB: Helped prepare challenge strain, conducted laboratory assays and analysis, provided data for manuscript, approved manuscript.

DE: Coordinated study, gave feedback on paper.

RF: helped with study coordination and clinical operational documentation

BD: Coordinated study, gave feedback on paper.

HW: performed laboratory assays for the study.

BF: Performed clinical assessments and clinical care of volunteers, gave feedback on the paper.

JH: Performed clinical assessments and clinical care of volunteers, gave feedback on the paper.

DS: Oversaw microbiology and immunology lab. Assisted with regulatory aspects. Served as advisor for study.


VGF: Oversaw trial from Sponsor perspective. Helped design study, analyze data and gave feedback on paper.