Phase 2b Study of the Safety, Immunogenicity, and Efficacy of a monovalent synthetic carbohydrate-based conjugate vaccine (SF2a-TT15) for protection against *Shigella flexneri* 2a experimental infection

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Sponsored by:
Karen L. Kotloff, MD

Vaccine Developer:
Institut Pasteur (Paris, France)

Funding by:
Institut Pasteur (Paris, France)

Principal Investigator:
Wilbur H. Chen, MD, MS

Clinical Site:
Center for Vaccine Development & Global Health
University of Maryland School of Medicine
Baltimore, MD 21201
U.S.A.

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Statement of Compliance

The study will be carried out in accordance with Good Clinical Practice (GCP) as required by the following:

- U.S. Code of Federal Regulations applicable to clinical studies (45 CFR Part 46, 21 CFR Part 50, 21 CFR Part 54, 21 CFR Part 56, and 21 CFR Part 312)
- International Conference on Harmonization (ICH) E6; 62 Federal Register 25691 (1997 and future revisions)
- The Health Insurance Portability and Accountability Act (HIPAA) Privacy Rule-Final Modification (45 CFR Parts 160 and 164)
- The local laws and regulations, which prevail over the University of Maryland, Baltimore
- Any terms of the award from the Bill & Melinda Gates Foundation

Compliance with these standards provides public assurance that the rights, safety, and well-being of study participants are protected, consistent with the principles that have their origin in the Declaration of Helsinki.

All key personnel (all individuals responsible for the conduct of this trial) have completed Human Subjects Protection Training.

SIGNATURE PAGE

The signature below constitutes the approval of this protocol and the attachments, and provides the necessary assurances that this trial will be conducted according to all stipulations of the protocol, including all statements regarding confidentiality, and according to local legal and regulatory requirements and applicable US federal regulations and ICH guidelines.

Site Investigator:

Signed: _____ Date: _____

Name
Title

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List of Abbreviations

AE	Adverse Event
ALT	Alanine aminotransferase
ALS	Antibody in Lymphocyte Supernatant
ANC	Absolute Neutrophil Count
ASC	Antibody Secreting Cell
CAPA	Corrective Action Preventative Action
CFR	Code of Federal Regulations
cfu	Colony Forming Units
CHIM	Controlled Human Infection Model
CIOMS	Council for International Organizations of Medical Sciences
CRO	Contract Research Organization
CTRIC	Clinical and Translational Research Informatics Center
CVD	Center for Vaccine Development & Global Health
DCF	Data Collection Form
DSMB	Data and Safety Monitoring Board
ECG	Electrocardiogram
FDA	Food and Drug Administration
FWA	Federal-Wide Assurance
GCP	Good Clinical Practice
GEMS	Global Enteric Multicenter Study
GMP	Good Manufacturing Practice
GMT	Geometric Mean Titer
Hg	Hemoglobin
HIPAA	Health Insurance Portability and Accountability Act
HLA	Human Leukocyte Antigen
HRPO	Human Research Protections Office
ICF	Informed Consent Form
ICH	International Conference on Harmonisation
ICMJE	International Committee of Medical Journal Editors
IDS	Investigational Drug Service
IEC	Independent or Institutional Ethics Committee
Ig	Immunoglobulin
IND	Investigational New Drug
IPA	Invasion Plasmid Antigen
IRB	Institutional Review Board
ISM	Independent Safety Monitor
LPS	Lipopolysaccharide
MCB	Master Cell Bank
MOP	Manual of Procedures
MPO	Myeloperoxidase

List of Abbreviations

MSD	Moderate-to-Severe Diarrhea
N	Number (typically refers to subjects)
NPP	Notification of Privacy Practices
NHP	Non-Human Primate
O-Ag	O-antigen
OHRP	Office for Human Research Protections
OPKA	Opsonphagocytic Killing Antibody
ORS	Oral Rehydration Solution
OS	Oligosaccharide
QA	Quality Assurance
QC	Quality Control
QM	Quality Management
QMP	Quality Management Plan
PBMC	Peripheral Blood Mononuclear Cell
PBS	Phosphate Buffered Saline
PI	Principal Investigator
PP	Per Protocol
RBC	Red Blood Cell
RU	Repeating unit
SAE	Serious Adverse Event
SBA	Serum Bactericidal Antibody
SF2a	*Shigella flexneri* 2a
SFC	Spot forming cells
SOP	Standard Operating Procedure
TAU	Tel Aviv University
TT	Tetanus toxoid
UMB	University of Maryland, Baltimore
UMMC	University of Maryland Medical Center
UP	Unanticipated Problem
USP	United States Pharmacopeia
WBC	White Blood Cell
WRAIR	Walter Reed Army Institute of Research
WHO	World Health Organization

Title: Phase 2b Study of the Safety, Immunogenicity, and Efficacy of a monovalent synthetic carbohydrate-based conjugate vaccine (SF2a-TT15) for protection against *Shigella flexneri* 2a experimental infection

Population: Approximately ninety healthy adults age 18-45 years from the Baltimore-Washington metropolitan area will be enrolled

Number of Sites: single site

Study Duration: 1.5 years

Subject Duration: 8 months, not inclusive of the screening period of up to 60 days

Study Products: Vaccine. **SF2a-TT15** is a prototype monovalent *Shigella flexneri* 2a vaccine, consisting of a synthetic pentadecasaccharide hapten corresponding to three repeating units of the *S. flexneri* 2a O-antigen and conjugated to tetanus toxoid. The product, ~1500 GMP vials, each containing 40 µg/mL of oligosaccharide was manufactured by Intravacc (Bilthoven, The Netherlands). The vaccine is to be diluted to the proper dose with the provided Alhydrogel adjuvant, and delivered intramuscularly.

Placebo. Sterile saline solution alone, delivered intramuscularly in blinded fashion.

Challenge Agent. Wild-type, live *S. flexneri* 2a strain 2457T is to be freshly harvested and diluted in sterile phosphate buffered saline to reach the desired inoculum.

Objectives:
Primary Objective:

- To assess the efficacy of SF2a-TT15 vaccination against Moderate-Severe Shigellosis Illness (*Appendix D*), as elicited by challenge with wild-type *S. flexneri* 2a strain 2457T.

Secondary Objectives:

1. To measure the safety and clinical tolerability of two sequential doses of SF2a-TT15
2. To evaluate the performance of efficacy of SF2a-TT15 vaccination against different case definitions and endpoint definitions (*Appendix D*), as elicited by challenge with wild-type *S. flexneri* 2a strain 2457T.
3. To evaluate the efficacy of SF2a-TT15 vaccination against any positive (qualitative) or quantitative fecal shedding of wild-type *S. flexneri* 2a.
4. To measure the serum IgG immune responses to SF2a LPS following vaccination and challenge.
5. To measure the bactericidal activity of SF2a-specific IgG following vaccination and challenge.
6. To measure the IgA and IgG antibody secreting cells (ASC) and antibody in lymphocyte supernatant (ALS) immune responses to SF2a LPS following vaccination and challenge.

Exploratory Objectives:

1. To measure pro-inflammatory cytokine markers in stools following challenge
2. To measure IgG subclasses (IgG1 and IgG2) to SF2a LPS following vaccination and challenge
3. To measure serum IgA immune responses to SF2a LPS following vaccination and challenge
4. To measure urinary secretory IgA to SF2a LPS following vaccination and challenge and to store urine samples for potential later analysis of anti-SF2a LPS IgG
5. To measure the IgA and IgG ASC expressing mucosal and other homing molecules (e.g., integrin α4β7-positive) to SF2a LPS following vaccination and challenge
6. To explore the correlates of immunity with protection
7. To compare the immunologic assays performed by different research laboratories
8. To collect, separate and store (at -70° C or colder) peripheral blood mononuclear cells (PBMC) so that in future studies the immune responses to SF2a-TT15 can be further characterized in great detail, including the measurement of memory, effector and other T cell subsets, B memory cells and other B cell subsets, expression of homing molecules, cytokine production and innate immune responses.
9. To collect and store serum, urine, and stool specimens for future studies, including but not limited to antibody microarray analysis, fecal 16S rRNA microbiome, transcriptomics, proteomics and other "omics".

Brief Description of Study Design:

This will be a phase 2b, double-blind, placebo-controlled, single-center study, involving a vaccination phase and a challenge phase. The vaccination phase will consist of study participants that will be 1:1 randomized to receive either the vaccine or placebo. Two doses of blinded study product will be given by intramuscular route of administration, separated by approximately 4 weeks. The challenge phase will consist of an inpatient stay of approximately 12 days during which eligible study participants will ingest an oral inoculum of wild-type *S flexneri* 2a strain 2457T and then be monitored for illness and treated with antibiotics when the primary endpoint is reached or upon 5 days post-challenge, whichever comes first, or when deemed necessary. Upon satisfying discharge criteria, study participants will complete outpatient clinic follow-up visits through ~7 months after last dose of blinded study product (Day 237). The efficacy study will be enrolled through three cohorts of participants, each cohort consisting of approximately 30 subjects that will be involved in the vaccination phase and 22 subjects that will proceed with the challenge phase.

Schematic of Study:

Vaccination Phase				
Cohort	Setting	No. doses	Target Enrollment	No. Subjects anticipated with 1:1 randomization

				SF2a-TT15	Placebo
1	Outpatient	2	30	15	15
2	Outpatient	2	30	15	15
3	Outpatient	2	30	15	15

Challenge Phase				
			No. Subjects expected to be challenged, by their 1:1 randomization	
Cohort	Setting	Target No. Challenge	SF2a-TT15	Placebo
1	Inpatient	22	11	11
2	Inpatient	22	11	11
3	Inpatient	22	11	11

1 KEY ROLES

Individuals: **Principal Investigator**
Wilbur H. Chen, MD, MS
Professor of Medicine
Direct Office Phone: 410-706-1188
Mobile Phone: 443-414-4462
wchen@som.umaryland.edu

CVD Immunology Collaborators
Marcelo B. Sztein, MD
Professor of Pediatrics, Medicine, and Microbiology & Immunology
Direct Office Phone: 410-706-2345
Mobile Phone: 443-538-1734
msztein@som.umaryland.edu

Marcela F. Pasetti, PhD
Professor of Pediatrics and Microbiology & Immunology
Direct Office Phone: 410-706-2341
mpasetti@som.umaryland.edu

CVD Microbiology Collaborator
Sharon M. Tennant, PhD
Associate Professor of Medicine
Direct Office Phone: 410-706-5336
stennant@som.umaryland.edu

Clinical Site: Center for Vaccine Development & Global Health
University of Maryland School of Medicine
685 W. Baltimore Street, Suite 480
Baltimore, MD 21201

Inpatient Research Isolation ward
Pharmaron, 5th floor
800 W. Baltimore Street
Baltimore, MD 21201

TAU Immunology Collaborator
Dani Cohen, PhD, MPH
Professor of Epidemiology and Preventive Medicine
School of Public Health, Faculty of Medicine
Tel Aviv University
Direct Office Phone: 972-3-6407081
dancohen@tauex.tau.ac.il

WRAIR Immunology Collaborator

LTC (P) Robert Cybulski, PhD
Director, Bacterial Diseases Branch
Walter Reed Army Institute of Research
Office Phone: 301-319-9511
robert.j.cybulski.mil@health.mil

Institut Pasteur

Christiane Gerke, PhD
Head of Vaccine Programs
Institut Pasteur
Paris, France
Office: +33 1 45 68 80 90
Cell Phone: +33 7 87 60 48 87
christiane.gerke@pasteur.fr

Mohand Ait-Ahmed
Project Manager
Center for Translational Research - Clinical Core
Institut Pasteur
28 rue du Docteur Roux
Paris, 75015, France
Phone : +33 1 44 38 93 02
Fax : + 33 1 40 61 39 77
Mohand.ait-ahmed@pasteur.fr

2 BACKGROUND INFORMATION AND SCIENTIFIC RATIONALE

2.1 Background Information

Diarrheal disease is a significant cause of morbidity and mortality worldwide; it is the second leading cause of death in children under the age of 5,[1] with approximately 212,000 deaths annually. In 2016, the global burden of *Shigella* was estimated to be the cause of 63,713 deaths in children under five years of age, predominantly in developing countries.[2,3] The Global Enteric Multicenter Study (GEMS) which investigated the cause of moderate-to-severe diarrhea (MSD) in children under 5 years of age in 7 sites in Africa and Asia identified *Shigella* among the top 4 pathogens.[1] GEMS follow-on studies using molecular diagnostic methods revealed an increased attributable burden due to *Shigella* and confirmed that it was the number 1 pathogen associated with MSD in the two older age strata (12-23 and 24-59 month olds) and fourth most important pathogen in the youngest 0-11 month old age group across all sites.[4,5] Moreover, *Shigella* was disproportionately associated with severe outcomes. In industrialized countries shigellosis, mostly *S. sonnei*, persists both as sporadic cases,[6] and as a problem in certain sub-populations such as children in daycare and other settings with suboptimal personal hygiene.[7] *Shigella* also causes significant disease in travelers, including military personnel, to endemic regions.[8,9]

The global public health importance of *Shigella* is further substantiated by additional factors. The increasing resistance to multiple antibiotics of *Shigella* isolates reduces therapeutic options.[7,10] The World Health Organization has named *Shigella* a priority for vaccine development and implementation but, despite intense efforts, no licensed vaccine to prevent *Shigella* diarrheal illness is widely available. *Shigella* is also classified as a category B agent of biodefense concern, making it a priority for development of therapeutics and vaccines.

2.1.1 Clinical Illness with *Shigella* Infection

After an incubation period of 1 to 4 days (as long as 8 days with *S. dysenteriae* [11,12]), there is fever and constitutional symptoms such as headache, malaise, anorexia, and occasional vomiting. Watery diarrhea typically precedes dysentery[11] and is often the sole clinical manifestation of mild infection.[13] Progression to frank dysentery may occur within hours to days with frequent small stools containing blood and mucus, accompanied by lower abdominal cramps and rectal tenesmus. Most episodes of shigellosis in otherwise healthy individuals spontaneously resolve within 5 to 7 days without sequelae. Antibiotic treatment hastens clinical and microbiological cure. With repeated infections by the same serotype, illness is absent or attenuated and excretion is diminished.[14-17]

A variety of extra-intestinal manifestations may occur. The most common is seizures, usually in febrile children without associated encephalopathy.[18] A rare complication of *S. dysenteriae* type 1 infection is hemolytic uremic syndrome.[19] *Shigella* sepsis is uncommon and is usually seen in hosts with malnutrition or immunodeficiency. Occasionally, *Shigella* causes focal extra-intestinal infections like meningitis,[20] arthritis,[21] splenic abscess,[22] and osteomyelitis,[23] in children. Persistent diarrhea and malnutrition are the most common chronic sequelae,[24] seen in children from developing countries. A rare post-infectious complication that occurs mostly in adults is a reactive inflammatory arthritis, alone[25] or as part of a constellation of arthritis, conjunctivitis or iritis, and urethritis.[26] This reactive arthritis is a spondyloarthropathic disorder characterized by

inflammation of the joints and tissues occurring after gastrointestinal or genitourinary infections, including: *Campylobacter*, *Salmonella*, and *Shigella*. Arthritis begins 2 to 4 weeks after the intestinal illness and the joint symptoms range from mild arthralgia to severe polyarthritis and may become chronic in about 10% of cases. Individuals with the human leukocyte antigen (HLA)-B27 histocompatibility antigen are predisposed, accounting for at least one-half of the cases.[27] The risk to the remaining 92-99% of the population that is HLA-B27 negative is extremely low.[28]

2.1.2 Antibody Responses to *Shigella*

Naturally-acquired wild-type *Shigella* infection confers serotype-specific immunity.[13,16,29] Moreover, adult subjects experimentally infected with either *S. sonnei* or *S. flexneri* are significantly protected against illness following re-challenge with the homologous strain (64-74% efficacy).[14,15] The specific immune responses that mediate protection against shigellosis have not been clearly elucidated. Several lines of evidence suggest an important role for anti-LPS antibodies. Israeli soldiers who lacked or had low titers of anti-LPS antibody were significantly more likely than seropositives to develop illness following exposure to the homologous *S. sonnei* or *S. flexneri* strain during military camp outbreaks.[29] Similarly, subjects who received bivalent *Salmonella* Typhi Ty21a-*S. sonnei* vaccine but lacked anti-LPS antibody were 3 to 7 times more likely to develop shigellosis following wild type *S. sonnei* challenge compared with seropositive vaccine recipients.[30]

Immune responses to *Shigella* plasmid-encoded outer membrane proteins (invasion plasmid antigens) IpaA, B, C and D and VirG are also encountered.[31] In contrast to the O antigen, these peptides are shared among the four serogroups; however, their role in conferring protective immunity remains uncertain.

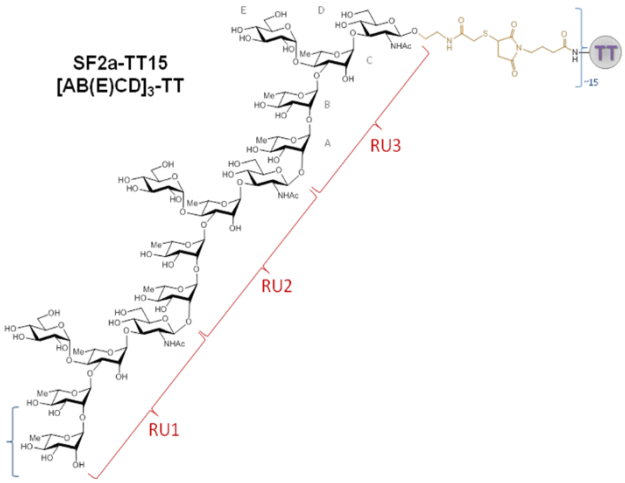
2.1.3 Polysaccharide Conjugate-Based Vaccine Development

A multitude of strategies have been pursued to develop vaccines against *Shigella* including live attenuated, killed whole cell, subunit, and conjugate vaccines.[32-34] Protective immunity against *Shigella* is directed against the LPS O-antigen (O-Ag) and is serogroup-specific and often serotype-specific.[35] Upon natural infection, experimental infection, or following vaccination, the antibody-mediated protection has been shown to be serotype specific, pointing to the O-Ag as the major protective antigen.

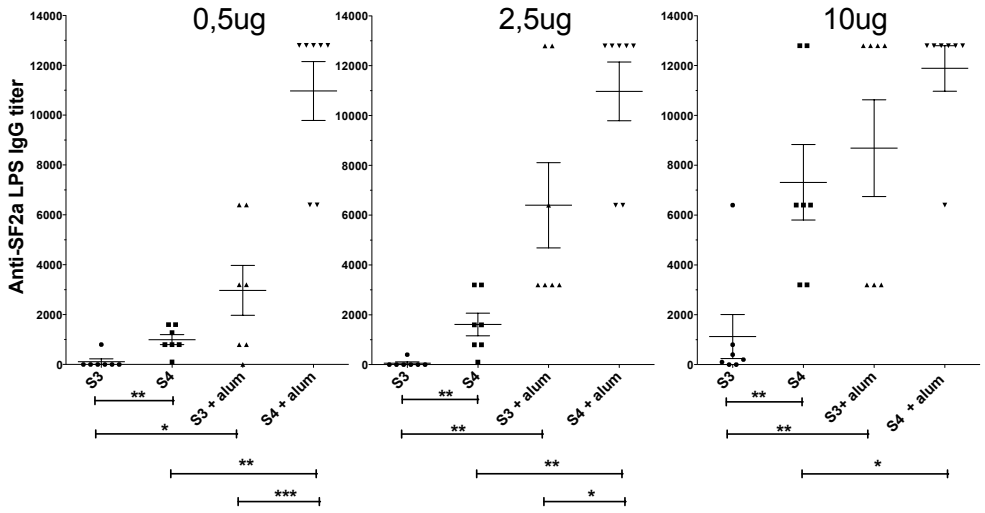
S. flexneri serotypes are defined by the structure of the oligosaccharide (OS) repeating unit (RU) that forms the O-Ag. For the predominant *S. flexneri* serotype 2a (SF2a), the biological RU is a branched pentasaccharide. Traditional glycoconjugate vaccines candidates are based on the use of detoxified LPS and present some drawbacks such as the need for accurate control of the LPS purification and detoxification steps and of the conjugation procedure to avoid impurities of biological origin and potential loss of immunogenicity upon chemical manipulation, including random conjugation to the protein carrier. An alternative strategy for the creation of a glycoconjugate vaccine is based on the use of chemically defined synthetic OSs identified as functional mimics of the natural O-Ag, which are covalently linked via single point attachment onto an appropriate carrier protein.

Rationally designed synthetic OSs of different lengths (di- to 20-mers) were chemically synthesized and covalently linked via single point attachment to a carrier protein, tetanus toxoid (TT). Upon testing their immunogenicity *in vivo*, the 15mer glycoconjugate vaccine candidate (Figure below) was identified as the optimal antigenic, conformational and structural mimic of the

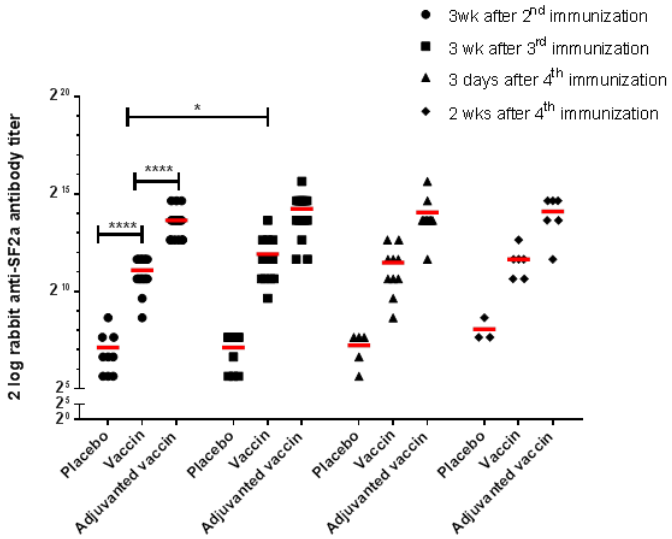
SF2a O-Ag. This 15mer glycoconjugate was also capable of eliciting a serotype-specific protective Ab response. The 15mer OS was also used in the form of a liposome-based diepitope immunogen and was observed to be a promising functional mimic of the SF2a O-Ag. Taken together, these data supported the advancement of the clinical development of the SF2a 15mer OS covalently linked to TT, also termed SF2a-TT15.



A pre-clinical GMP lot of SF2a-TT15 was evaluated for immunogenicity in BALB/c mice. Mice were immunized with SF2a-TT15 alone or with Alhydrogel adjuvant (aluminum hydroxide, Brenntag, Denmark). Three sequential intramuscular doses of 0.5 µg, 2.5 µg, or 10 µg by OS weight was administered every 3 weeks, and a fourth booster dose was administered 1 month followed the third dose. The serum anti-SF2a LPS IgG ELISA titers were measured after the third and fourth doses of vaccine. As shown in the Figure below, whatever the dose tested, there was a significant increase of the IgG titer when administered with Alhydrogel. The 0.5 µg and 2.5 µg dose, but not the 10 µg dose, in the presence of Alhydrogel also gave rise to significant increases in the IgG titer when comparing the 3 and 4 sequential dose immunization series. Together, these results indicate that it is possible to decrease the dose of the vaccine candidate to be used when adjuvanted with Alhydrogel.. These findings also justified the use of Alhydrogel and of 2 different doses of our vaccine candidate in the Phase 1 clinical trial.



A GLP animal toxicity study with the pre-GMP SF2a-TT15 alone or adjuvanted with Alhydrogel was conducted. A total of 48 male and female New Zealand White albino rabbits were immunized with four intramuscular doses of 10 µg SF2a-TT15 alone, 10 µg SF2a-TT15 with Alhydrogel, or placebo (saline) at 3 week intervals. The scheduled blood sampling for clinical laboratory analysis and necropsy examinations did not demonstrate abnormalities—so the vaccine with or without adjuvant was considered well-tolerated in rabbits. Serum anti-*S. flexneri* 2a LPS IgG ELISA antibodies were measured. A significant increase in these specific antibodies was observed among animals that received SF2a-TT15 with Alhydrogel adjuvant and a plateau was achieved after 2 doses.



A first-in-human, phase 1 clinical trial was performed in Israel, at the Tel Aviv Sourasky Medical Center and Tel Aviv University with a GMP formulation of SF2a-TT15. Sixty four eligible participants were screened for low pre-existing *S. flexneri* 2a LPS antibody levels and HLA-B27 seronegativity and randomized to receive vaccine alone, vaccine with Alhydrogel adjuvant, or placebo. The vaccine was evaluated at 2 µg and 10 µg doses. Three sequential injections of the blinded study product were administered by intramuscular route at 1 month intervals and follow-up was through 3 months after the last dose of vaccine. The SF2a-TT15 vaccine, with or without Alhydrogel, was well-tolerated at both the dosage levels (2 µg and 10 µg doses). With 2 µg doses, the first vaccine dose induced significant rises in serum anti-*S. flexneri* 2a LPS IgG, with geometric mean titers (GMT) of ~5-fold over baseline or compared to placebo recipients. Alhydrogel adjuvanted doses enhanced these serum antibody responses after the second and third dose of vaccine with ~8.5 and ~12-fold increases in GMT. The first dose of the 10 µg dose vaccine elicited GMT fold increases over baseline of ~25-fold and ~27-fold when administered with or without Alhydrogel; there was no further detectable increase subsequent to the second and third dose of vaccine (adjuvanted or non-adjuvanted). However, the peak level of the antibody response was maintained longer if the vaccine was administered with Alhydrogel. All vaccinees receiving the 10 µg dose (with or without Alhydrogel) were responders after the first dose of vaccine (responses defined as 4-fold or greater increases in antibody titer over baseline). Furthermore, vaccination induced ~15-fold increases in serum bactericidal antibody (range of post-vaccination GMT 5918-14669); there was an 88% response rate (4-fold or greater increase) in serum bactericidal titer after the first dose of vaccine. In conclusion, these results support the further clinical development of the SF2a-TT15 vaccine candidate at the 10 µg dose, delivered with Alhydrogel adjuvant, in a

one or two-dose regimen.[36]

2.2 Rationale

Since 1992, 3973 volunteers (974 adults and 2999 children aged 1-7 years) have received first generation *Shigella* conjugate vaccine candidates, including *S. dysenteriae* 1, *S. sonnei* and *S. flexneri 2a* conjugate vaccines manufactured at the National Institutes of Health using traditional chemical O-PS extraction and conjugation techniques.

These investigational *Shigella* conjugate vaccines showed a very good safety profile similar to the other conjugates licensed and widely used in national immunization programs all over the world. The serum immune response was stronger and longer lasting than that induced by natural infection. The *S. sonnei* conjugate showed 74% protective efficacy in young adults[37] and 71% protective efficacy in children aged 3-4 years but was not protective in younger children.[38] The levels of serum IgG anti-LPS antibodies to *S. sonnei* induced by the conjugate vaccine correlated with protection against natural infection. Improved conjugates are needed to be more immunogenic and confer protection in children less than 3 years of age.

The SF2a-TT15 glycoconjugate vaccine candidate represents the next-generation of glucoconjugate *Shigella* vaccine. It has been developed by Institut Pasteur (Paris, France), a French non-profit foundation which has a long-standing history of developing vaccines. The safety and immunogenicity of SF2a-TT15 in a phase 1 trial in Israel described above and supports continued clinical development.

The intent of the planned study is to evaluate the efficacy of SF2a-TT15 using a controlled human infection model (CHIM). *Shigella* is a human-specific pathogen and no predictive animal model resembling human disease is available. While immunogenicity is assessed in animal models, efficacy testing in animals against intestinal disease similar to what occurs in humans is not feasible. Non-human primates (NHP) in captivity can be infected following very high doses, but these unintentional exposures limit the validity of this model for humans. In humans, the infectious dose can be as low as 10 bacteria. In NHP, a dose of 10^{10} bacteria is required to cause disease. Thus, the pre-clinical animal models which are available are so different from human infection, making meaningful efficacy testing with these models impossible. Therefore, a controlled human infection model (CHIM) has been in use for more than 50 years to obtain early efficacy read-outs for *Shigella* vaccine candidates as an important decision point for further development and to identify the most promising candidates for further development. Only if protection is observed following vaccination and the CHIM will the SF2a-TT15 candidate be further developed into a multivalent *Shigella* vaccine and undergo clinical testing to Phase III efficacy trials. The absence of demonstrable protection in the CHIM would prevent performing large efficacy trials in young children and infants with a vaccine with little chances of success.

The wild-type *S. flexneri* 2a strain 2457T challenge model has been in use for many decades, ans was first developed at the University of Maryland School of Medicine.[11] A modern challenge model with 2457T has been in use since the early 1990s[15] and is anticipated to elicit 80-90% attack rates of illness according to the case definition employed among naïve study subjects that ingest ~1500 cfu organisms.

To harmonize CHIM studies for *Shigella* and thus allow for indirect comparisons of various *Shigella* vaccine candidates, the Bill & Melinda Gates Foundation (BMGF) organized a working group to define efficacy endpoints and immunological assays for guiding the scientific community (personal communication with C. MacLennan, BMGF). These modified efficacy endpoints reflect

moderate and severe shigellosis. Protection against these forms of shigellosis is currently deemed the most desirable feature of a successful vaccine. A post-hoc analysis of published studies was performed to determine the attack rate for these endpoints and an attack rate of 60% was estimated (Chad Porter, VASE 2018).

Assuming an attack rate of 60% among placebo controls, 33 subjects per group (vaccinees and controls) are required at 80% power to demonstrate 50% efficacy, with the lower limit of the 90% confidence interval larger than zero. Thus, 66 subjects will be randomized to receive vaccine or placebo and enrolled in the CHIM to evaluate the efficacy of SF2a-TT15. Based on local clinical site facility and staff resources, the 66 subjects will be split into 3 cohorts of 22 subjects each. To account for potential drop outs between the vaccination and CHIM phase and to plan for back-ups, up to 30 volunteers per cohort will be enrolled in the vaccination phase, thus a total of 90 subjects are targeted for enrollment. The excess of 8 subjects per cohort is based on experience of the Center for Vaccine Development (CVD) with previous human challenge studies.

2.3 Potential Risks and Benefits

This clinical trial will involve the use of non-licensed vaccines and human experimental infections (challenges) with a wild-type *Shigella* organism. Therefore the design and implementation of this trial has been planned with the intent to minimize risk, pain, and discomfort, whenever possible. The sample size of the study has been calculated to expose as few persons as possible to the challenges (the part of the study which carries the most potential risk). The challenges will be conducted under containment and the discharge of participants will only be done when they have demonstrated they no longer shed the organism, so that the risk for the pathogen to cause infections in community contacts beyond the inpatient ward has been minimized. Objective management of fluid losses and illness related to shigellosis are carefully described in the protocol (Section 5.6) and Manual of Procedures (MOP). Early treatment with antibiotics will be initiated as necessary to lessen the duration and severity of shigellosis.

2.3.1 Potential Risks

Anticipated Reactions with an Injectable Conjugate Vaccine. Local injection site pain, swelling, tenderness, and warmth is anticipated, but is expected to be mild, of short duration, and will self-resolve. Parenteral vaccines occasionally can also cause constitutional symptoms such as fever, aches, malaise, or anorexia. As part of the objective to continue to measure the safety and potential reactogenicity of the vaccine, the study will measure the local and systemic reactogenicity and will document unsolicited adverse events (AEs) which may be related to vaccination. Additional potential hypersensitivity reactions to the vaccine are expected to be rare and unpredictable. Persons who have a history of anaphylaxis with tetanus containing vaccines will be excluded from participation.

Shigellosis and Diarrheal Disease. The *Shigella* challenge is intended to elicit symptoms consistent with shigellosis, including fever, headache, diarrhea, dysentery, abdominal cramps, tenesmus, nausea, and vomiting. If a subject were to develop unacceptable symptoms as described above he/she will be offered early antibiotic therapy. Antipyretics and non-steroidal anti-inflammatory pain medications can be provided to alleviate some of the discomfort associated with shigellosis. The potential exists for *S. flexneri* strains to cause a dehydrating diarrheal disease. Under the controlled management in this study, the dehydrating effects of heavy purging can be prevented by giving oral rehydration solutions preventively after each loose stool (grade

3-5) and by early recognition of dehydration and prompt therapy with oral and intravenous fluids. If intravenous fluids are required, blood chemistries will be monitored. As a precaution, subjects who do not have prominent arm veins will be excluded from participating in the trial because this may pose difficulty in attaining venous access.

Reactive Arthritis. A rare post-infectious complication that occurs mostly in adults who have had an infection with a wild type *S. flexneri* serotype is reactive inflammatory arthritis, alone[25] or as part of a constellation of arthritis, conjunctivitis or iritis (uveitis), and urethritis.[26] Arthritis begins acutely usually 2 to 4 weeks after the intestinal illness. Joint symptoms range from mild arthralgia to severe polyarthritis and become chronic in about 10% of cases. Persons with the HLA-B27 histocompatibility antigen are predisposed, accounting for ~half of the cases.[27] The risk to the remaining 92-99% of the population that is HLA-B27 negative is thus extremely low. Subjects who carry HLA-B27 will be excluded from participating in the study because of the remote possibility of inducing this arthritis syndrome. Uveitis can be a component of the syndrome (which has many causes), but to our knowledge has never been associated with *Shigella* infection. Uveitis in association with reactive inflammatory arthritis can cause pain, eye redness, and visual disturbance. Complete recovery within 3 months is the usual outcome.

Bacteremia and/or Localized Infection. Even during infection with virulent *Shigella* spp., available information suggests that bacteremia and localized infection are rare in persons of good nutritional state and among adults. Therefore, this theoretic risk is not specifically discussed in the consent form.

Transmission and Secondary Spread of the Live Organism. The transmission of this organism is through the fecal-oral route. Subjects will be repeatedly educated during the course of the study about precautions (e.g., hygiene and toilet cleaning procedures) needed to prevent spread of the organism to close contacts. Hand washing will be emphasized to the subjects. Stools will be treated with bleach for five minutes prior to flushing. Previous inpatient studies with wild type *S. flexneri* 2a at the CVD have not detected transmission events. To prevent the transmission of the organism outside of the research isolation ward, subjects must complete antibiotic therapy and lack the shedding of organisms in stools cultures prior to discharge.

Hypersensitivity and/or Side Effects to Antibiotics. Subjects with known hypersensitivity to ciprofloxacin or trimethoprim-sulfamethoxazole will be excluded from the study. These licensed antibiotics have been widely used and have an excellent safety record; however, there remains a small risk that a hypersensitivity reaction to the licensed antibiotic could occur, and in rare instances such reactions have been fatal. The following reactions are seen rarely in persons taking ciprofloxacin: 1) gastrointestinal (GI) upset (e.g., upset stomach, nausea, vomiting, diarrhea); 2) headache, dizziness, or light-headedness; 3) seizures (therefore subjects with a known seizure disorder may not participate); and 4) photosensitivity (so subjects are counseled to avoid such exposure); and 5) tendinitis (pain, inflammation, or ruptured tendon). Other reactions may occur from drug interactions, including caffeine, so subjects are not allowed to take any other medications which may interact with ciprofloxacin while they are participating in this trial, and are advised to avoid caffeinated beverages while taking ciprofloxacin. Uncommonly, reactions are seen with trimethoprim/sulfamethoxazole including: GI upset, hypersensitivity, hepatitis, and blood dyscrasia. These reactions are rare but can occasionally be fatal.

Pregnancy. The vaccine has not been evaluated in pregnancy, so pregnant women may not participate in the study and women of child-bearing potential must agree to use effective methods of birth control for at least 4 week prior to enrollment and 4 weeks after last dose of vaccination

or challenge. Women will be counseled about the pregnancy precautions related to study participation.

Blood Draws. Blood drawing may cause local pain, bruising, occasional lightheadedness, and rarely fainting. Blood will be drawn by trained personnel. Subjects who are found to experience lightheadedness will lie down during and after they have their blood drawn. The amount of blood drawn does not exceed that recommended by the American Red Cross.

Confidentiality. Personal health information will be collected as a part of this study and efforts will be made to maintain confidentiality. There is a small risk of loss of confidentiality that an unauthorized person may gain access to viewing the research records. In order to maintain confidentiality, all study records will be stored in a secure location, such as a locked office and/or locked cabinet. Electronic data will be password-protected. Study records and specimens obtained will be coded. Research records will only be shared with authorized personnel and only in connection with carrying out the obligations relating to the study. Every effort will be made to keep the records as confidential as possible, within the limits of the law.

Unexpected Risks. There may be other side effects, including severe ones, which cannot be predicted.

Special Circumstance for COVID-19. As long as the threat of COVID-19 continues to represent a U.S. Public Health Emergency, a number of steps will be undertaken to protect research staff and study participants from COVID-19. The eligibility criteria will include a question regarding the presence of symptomatic COVID-19-like illness, wherein a positive response of symptoms will exclude the participant from eligibility to be vaccinated. Also, all participants must have completed COVID-19 vaccination at least 14 days prior to challenge and each participant must test negative from a molecular diagnostic test prior to challenge. In order to separate the anticipated side effects of COVID-19 vaccination from the evaluation of the reactogenicity of the blinded study product, COVID-19 vaccines must not be administered within <14 days of scheduled blinded study product administration. These measures are intended to further reduce the risk of transmission of COVID-19 on the inpatient setting from asymptomatic or pre-symptomatic participants.

Furthermore, at all points during the conduct of the study, a number of non-pharmacologic interventions will be required. The observance of universal masking, physical distancing, hand hygiene, administrative changes (e.g., decreasing the density of study personnel), and other control or mitigation measures will be implemented by research staff, as per the university policies. All study participants will be required to wear a face mask and instructed to perform hand washing or hand sanitation during the inpatient period and for all scheduled outpatient visits.

When the COVID-19 pandemic resolves and the U.S. Public Health Emergency has ended, the study team will re-evaluate the local conditions for consideration of removing these additional precautionary measures.

2.3.2 Known Potential Benefits

This is a healthy volunteer study which does not provide any guarantee of benefit. The benefit is largely the scientific knowledge to be gained from the study, which will be an essential component of the development of a new vaccine, which may benefit many people and prevent illness and death in many developing countries.[39,40] The development of *Shigella* vaccines has long been impeded[32,41,42] and there are no suitable animal models which can replace human studies.[43]

There is the prospect that the study vaccine may confer protection against *S. flexneri* 2a infection,

but the study is blinded and includes a control vaccine, thus this benefit is not guaranteed. There is no approved vaccine for *Shigella* in the U.S.

Participants will be provided access to the general results of the study, as these data are required to be made available through clinicaltrials.gov registry. Participants may know, upon their request, their randomized vaccination group assignment; this will occur upon the closure of the study, locking of the data base, and unblinding of the study results.

3 STUDY DESIGN

This is a phase 2b, randomized, double-blind, placebo-controlled, single-center study of the safety, immunogenicity, and preliminary efficacy of SF2a-TT15. We hypothesize that the synthetic glycoconjugate *Shigella* vaccine will demonstrate protection in a human volunteer challenge model using wild-type *S. flexneri* 2a strain 2457T. The study will consist of two phases, a vaccination phase and a challenge (CHIM) phase. The vaccination phase begins when volunteers who meet eligibility criteria following a screening process are enrolled and randomized to receive two doses of either vaccine or placebo in a blinded fashion, with each dose separated by approximately 4 weeks. Volunteers complete an outpatient clinic follow-up visit approximately one week after each dose. The target of the study is to vaccinate 90 volunteers of whom 66 will undergo CHIM; approximately half (n=33) of the CHIM participants will have received 2 doses of the vaccine and the other half will have received 2 doses of placebo. The logistical implementation of the study will be conducted with three cohorts of participants involved in the vaccination and subsequent challenge phases of the study.

Eligible and available consenting volunteers in cohorts 1-3 who have completed the vaccination phase will be eligible for the challenge phase, which includes an approximate 12-day inpatient stay. In order to ensure the target number of participants will complete the challenge phase, for the primary objective of the assessment of efficacy, there is a plan to have additional volunteers available for participating in the challenge phase. For example, in order to meet a target of n=22 challenged in a single cohort, there will be up to 30 participants enrolled into the vaccination phase of that cohort and there may be up to 26 eligible participants admitted to the inpatient ward during the acclimatization period (prior to challenge). Thus 8 of these participants will serve as “back-ups” (8 of 30 persons per cohort that will not be challenged), the identity of which participants are back-ups is blinded to the subject. An unblinded statistician will review the list of volunteers identified for challenge and for homing studies to ensure the proportion of vaccine and placebo recipients is balanced; when necessary, the unblinded statistician may recommend alternative study participants (among the back-ups) to ensure this balance. Back-up participants (participants on the inpatient ward during the acclimatization period who have not been challenged) are immediately discharged from the inpatient unit.

The challenge phase is located in an inpatient setting (Research Isolation Ward), during which time volunteers will be inoculated with the wild-type *Shigella* (*S. flexneri* 2a strain 2457T). All participants will be monitored for the onset of illness and actively managed to treat their symptoms. In order to be eligible for discharge, the participant must complete a course of antibiotics, demonstrate no symptoms of illness, and provide two stool specimens which fail to grow *S. flexneri* 2a, the challenge organism.

All participants, whether participating in the challenge or not, will continued to be followed through approximately 7 months after the last dose of vaccine for the collection of safety information.

Plasmablast homing studies (ASC and ASC homing studies) will be performed to identify the mucosal or systemic distribution of primed cells. Because these studies are labor-intensive, they will be performed on a subset of up to 6 participants maximum per day (or up to 8 participants/cohort)—each cohort is intended to be vaccinated on 2 separate days. We anticipate that up to 24 volunteers in the vaccination phase and 18 volunteers in the challenge phase will participate in homing studies. It is intended that the same participants in the homing studies for the vaccination phase be the same 18 participants in the homing studies during the challenge phase, therefore, in order to meet the number in the challenge phase, we plan to have more

participants (up to 24) in the homing studies for the vaccination phase. Participants in the homing studies will be selected somewhat randomly since there will be an attempt to maintain both the blind and also the balance of vaccine and placebo recipients; priority for challenge will be given to homing study participants.

4 STUDY POPULATION

4.1 Study Population

Healthy subjects, ages 18-45 years, will be recruited from the Baltimore-Washington area on the basis of general good health, expressed interest in the study as determined at a preliminary interview, satisfying all eligibility criteria, and availability for the challenge and required follow-up visits. Approximately 90 subjects will be recruited and vaccinated, with the aim of challenging 66 subjects. Subjects must undergo medical screening and meet eligibility criteria within 60 days before vaccination.

4.2 Inclusion/Exclusion Criteria

Inclusion Criteria:

1. Male or female of age 18-45 years
2. Provides written informed consent
3. Healthy, based on history, exam, and medications
4. Documented acceptable screening laboratory work, including:
    - WBC, ANC, Hg, Platelets
    - Creatinine, ALT, Bili
    - Serum IgA
    - HIV, HBsAg, HCV
    - Negative for HLA-B27
    - urinalysis
5. Passing score on *Comprehension Assessment Tool* (≥70% correct answers)
6. Agrees not to participate in another interventional clinical trial during the study period (approximately eight months)
7. For females of child-bearing potential†, must agree to acceptable birth control*, 4 weeks before enrollment and through 4 weeks after last vaccination or challenge

    † *females of child-bearing potential are defined as: not sterilized via tubal ligation, bilateral oophorectomy, salpingectomy, hysterectomy, or successful Essure® placement (permanent, non-surgical, non-hormonal sterilization) with documented radiological confirmation test at least 90 days after the procedure, and still menstruating or <1 year of the last menses if menopausal.*

    * *acceptable birth control includes: non-male sexual relationships, abstinence from sexual intercourse with a male partner, monogamous relationship with vasectomized partner who has been vasectomized for 180 days or more prior to the subject receiving the first study vaccination, barrier methods such as condoms or diaphragms with spermicide or foam, effective intrauterine devices, NuvaRing®, and licensed hormonal methods such as implants, injectables, or oral contraceptives.*
8. Available for a 12-day inpatient stay
9. During the time when a U.S. Public Health Emergency for COVID-19 exists, the participant must have completed an FDA EUA authorized COVID-19 vaccination, at least 14 days prior to challenge.

Exclusion Criteria:

1. Positive pregnancy test at screening or within 24h of study product dosing
2. Poor venous access, as defined by inability to obtain venous blood after 3 venipuncture attempts
3. Abnormal vital signs, defined as:
   - Systolic BP>150 mmHg or Diastolic BP>90 mmHg
   - Resting heart rate >100
   - Oral temperature ≥100.4°F
4. Persons with IgA deficiency (serum IgA less than lower limit of reference range)
5. Serum *S. flexneri* 2a LPS IgG titer of >2500
6. Received prior vaccines or had prior infection (natural or challenge) with ETEC or *Shigella*, within 5 years prior to enrollment
7. Received a COVID-19 vaccine within <14 day prior to enrollment (dose #1 of blinded study product), within <14 days prior to dose #2 of blinded study product, or within <14 days prior to challenge
8. Symptoms of Traveler's diarrhea associated with travel to countries where *Shigella* or other enteric infections are endemic (most of the developing world) within 3 years prior to enrollment
9. History of chronic gastrointestinal illness, including severe dyspepsia, or other significant gastrointestinal tract disease
10. Use of antibacterials within 2 weeks of each dose of vaccine or the challenge
11. Regular use (≥weekly) of laxatives, anti-diarrheal agents, anti-constipation agents, or antacid therapies
12. History of major gastrointestinal surgery (uncomplicated laparoscopic appendectomy or cholecystectomy >1 year prior is permitted)
13. Abnormal bowel pattern, defined by <3 stools per week or >2 stools per day in the past 6 months
14. Use of oral, parenteral or high-dose inhaled steroids† within 30 days of each dose of vaccine or the challenge
    † *high-dose oral steroids is defined as prednisone ≥ 20 mg total daily dose, or equivalent dose of other glucocorticoids; high-dose inhaled steroids is defined as >800 µg/day of beclomethasone dipropionate or equivalent*
15. Use of any medication which might affect immune function* within 30 days of each dose of vaccine or the challenge
    * *examples include: anti-cancer chemotherapy, immunomodulatory medications for rheumatic diseases, and anti-rejection transplant medications*
16. Current medical condition which requires more than a single daily (2 or more) prescribed medication for control (as medication use is limited during the inpatient stay). *In other words, a subject may be eligible if on a single medication for a stable, well-controlled medical condition which has not required any changes in dose or frequency over at least 3 months. Any change in prescription due to a change in provider, insurance, financial reasons, etc. and as long as the change in prescription remains in the same class of*

medication will not be considered an ineligibility (is allowable towards the definition of a stable, well-controlled medical condition). Any change in prescription medication due to an improvement of a condition will also not be considered an ineligibility. Under all circumstances, the medical condition which is being treated with the single medication must be reviewed and deemed to be acceptable by the judgement of the investigator. The medical condition must not pose a significant risk to the participant nor to the safe conduct of the study.

17. History of reactive arthritis
18. Diagnosis of schizophrenia or other major psychiatric disease
19. History of seizure disorder within the last 5 years
20. History of alcohol or drug abuse within last 5 years
21. Presence of immunosuppression
22. Known significant allergy (i.e., anaphylaxis) to ciprofloxacin, trimethoprim-sulfamethoxazole (Bactrim), or a tetanus-containing vaccine
23. 12-lead electrocardiogram with pathologic abnormalities (see Appendix B)
24. Occupation in food handling industry, living with, or care of very young children (<5 years old), elderly (>65 years), or immunocompromised
25. Having any known legal obligations, court appearances, professional meetings, vacations, planned events, or other reasons which might interfere with a person's availability for a 12-day inpatient stay.
26. During the time when a U.S. Public Health Emergency for COVID-19 exists, on the days of vaccination there is the presence of 2 or more of any of the following symptoms: fever (≥38°C), chills, myalgia, headache, sore throat, or new olfactory and/or taste disorder
27. Any other criteria which, in the investigator's opinion, would compromise the safety of the study, the ability of a subject to participate, or the results of the study

4.3 Enrollment and Randomization Procedures

Per International Conference on Harmonisation (ICH) guideline E6: Good Clinical Practice (GCP), screening records will be kept at the clinical site to document the reason why an individual was screened, but failed trial entry criteria. The reasons why individuals failed screening will be recorded in the data system.

Based on local clinical site facility and staff resources, subjects will be enrolled in 3 cohorts, each cohort consisting of up to 30 subjects. Once consented and upon confirmation of eligibility for this trial, the subject will be enrolled and randomized. Subjects will be randomized, within their respective cohort, in a 1:1 ratio to receive either vaccine or placebo. The randomization code will be prepared by a statistician at the CVD. Within each cohort (n=30), randomization will be generated by computer to allocate 15 participants to each group (vaccine or placebo) using block randomization with varying block sizes 6, 8, 10 or 12. A designated individual at the site will be provided with a code list for emergency unblinding purposes, which will be kept in a secure place.

Subjects who sign the informed consent form and are enrolled and randomized but do not receive study vaccine may be replaced. Subjects who sign the informed consent form, and are randomized and receive at least one dose of vaccine but subsequently withdraw, or are withdrawn, or are terminated from this trial, or are lost to follow-up will not be replaced.

Subjects that have completed 2 doses of blinded study product will be eligible for the challenge phase. Subjects that do not complete 2 doses of blinded study product and subjects who are determined to be ineligible or inappropriate for the challenge will not be replaced.

4.4 Withdrawal from Study

Reasons for Withdrawal or Termination. Participation in the study is strictly voluntary. Participants have the right to withdraw from the study at any time and for any reason, without penalty or prejudice to further treatment. The Principal Investigator (PI) and/or designee may, at their own discretion, terminate a subject from continuing in the study if it is considered to be in the participant's best welfare, or if the participant is not willing or able to comply with the study requirements. The reason for withdrawal or termination will be documented.

There may be participants that will be withdrawn from the challenge phase of the study, after successfully completing the vaccination phase. It is anticipated that some participants could be withdrawn due to inappropriate behavior or other unacceptable issues, which could present a risk to the participant, other subjects, the study staff, and/or jeopardize the conduct of the study. Participants may also withdraw from the study for any reason, even during the inpatient confinement. These participants will be discharged from the inpatient unit (if present on the inpatient unit at the time of this determination) and in the presence of a security guard, when applicable. If the participant withdraws or is being removed after being challenged and prior to completion of all scheduled doses of antibiotics, then a 1 gram dose of ciprofloxacin (i.e., double oral dose or equivalent for another antibiotic) will be administered prior to discharged, to reduce the risk of infectiousness to the general community; remaining doses to complete a total of 3 days of therapy will be provided to the participant prior to "early discharge". Instructions for outpatient follow-up will be provided at the time of "early discharge", with the intent to continue all protocol-described outpatient visits, for the purpose of collecting safety information and the collection of research specimens. Participants will ultimately decide on what amount of follow-up and safety or research specimens can/will be collected, upon their withdrawal or their decision to terminate participation in the study.

Handling of Participant Withdrawal or Termination. Every effort will be made to undertake protocol-specified safety follow-up procedures to capture AEs, serious adverse events (SAEs), and unanticipated problems (UPs). In the event of withdrawal or termination from the study, reasonable efforts should be made to conduct the following procedures:

- Review diary card/ memory aid if still in use prior to withdrawal
- Update any ongoing AE/SAEs that remain ongoing at time of subject's last visit prior to withdrawal
- Query about AEs, SAEs and concomitant medications if the interval between the subject's last visit and the time of withdrawal is within the protocol defined reporting period
- Perform physical examination
- Obtain blood for safety laboratory testing if withdrawal occurs before the day 36 visit
- Update contact information

Premature Termination or Suspension of Study. This study may be temporarily suspended or prematurely terminated if there is sufficient reasonable cause. Written notification, documenting the reason for study suspension or termination, will be provided by the suspending or terminating party to the PI, University of Maryland Baltimore Institutional Review Board (UMB IRB), Institut Pasteur, funding agency (BMGF), independent data and safety monitoring board (DSMB), and

the U.S. FDA (as the regulatory authority). If the study is prematurely terminated or suspended, the PI will promptly inform the IRB and will provide the reason(s) for the termination or suspension. Circumstances that may warrant termination or suspension include, but are not limited to:

- Determination of unexpected, significant, or unacceptable risk to participants
- Demonstration of clear efficacy that would warrant stopping early (e.g., if there is near 100% efficacy in the first two cohort challenges, then the third cohort could be suspended)
- Insufficient compliance to protocol requirements
- Data that are not sufficiently complete and/or evaluable (e.g., if loses were so excessive that the target number of challenges cannot be achieved in the three planned cohorts)
- Determination of futility

The study may resume once concerns about safety, protocol compliance, and data quality are addressed and satisfy the IRB, DSMB, and FDA.

5 STUDY PRODUCTS

5.1 Study Vaccine, SF2a-TT15

The key component in SF2a-TT15 is the fully synthetic OS made of 15 monosaccharide residues, corresponding to three non O-acetylated biological RUs of the *S. flexneri* 2a O-Ag. It is therefore named either 15mer, Sf2aOS3, or [**AB**(**E**)**CD**]$_3$, whereby **A**, **B** and **C** are α-linked L-rhamnopyranosyl residues, **D** is a β-linked 2-*N*-acetyl-2-deoxy-D-glucopyranosyl residue (*N*-acetyl-D-glucosamine) and **E** is an α-linked D-glucopyranosyl residue. The conjugation chemistry used for the synthesis of SF2a-TT15 involves the construction of a thioether linkage between the spacer-equipped 15mer OS and a maleimide-activated TT (TT_{Mal}). The manufacturing, including all the conjugation chemistry, was performed under GMP conditions at Intravacc (Bilthoven, the Netherlands).

One dose (0.5 mL) of vaccine contains after dilution with aluminum hydroxide adjuvant (Alhydrogel®, Brenntag, Denmark) the following components:

- SF2a synthetic 15mer OS, 8.8 µg
- Tetanus toxoid, 29.1 µg
- Al^{3+}, 0.680 mg
- Excipients: Tris(hydroxymethyl)aminomethene, Sodium chloride, and water for injection

The SF2a-TT15 vaccine is to be stored at 2-8 °C, do not freeze. The SF2a-TT15 vaccine (0.5 mL) and adjuvant (0.8 mL) are filled in 3-mL hydrolytic type 1 glass (Ph.Eur) vials, sealed with a bromobutyl rubber stopper, and an aluminum crimp cap with polypropylene flip-off cap. The SF2a-TT15 vaccine is a clear colorless liquid and the adjuvant is a white turbid liquid after homogenization. The SF2a-TT15 vaccines are packed as a kit with the adjuvant vial.

5.1.1 Preparation of Vaccine Doses for Administration

The final study product for administration is obtained by mixing 0.5 mL of adjuvant with 0.5 mL of SF2a-TT15 vaccine. Adjuvant will be drawn up with a disposable syringe and added to the vial with SF2a-TT15, then the diluted SF2a-TT15 vaccine must be shaken gently for 1 minute before use. A 0.5 mL volume of the diluted SF2a-TT15 vaccine will be drawn up to a separate disposable syringe for intramuscular administration. The diluted study product vial can be used up to 4 hours, if stored at 2-8 °C, or up to a maximum of 30 minutes if kept at room temperature. Prepared diluted vaccine must be manually shaken gently before using. Once the mixed vaccine has been drawn into a syringe, it should be administered immediately (within 30 minutes). Any unused vaccine or waste material should be disposed of in accordance with local requirements.

5.2 Placebo, Sterile Saline

The placebo is commercially-available physiologic sterile saline.

5.2.1 Preparation of Placebo Doses for Administration

The masked placebo consists of 0.9% sterile Sodium Chloride (normal saline), United States Pharmacopeia (USP) grade. The saline vials are to be stored at room temperature

(20-25 °C). A 0.5 mL volume of normal saline will be drawn into a disposable syringe for intramuscular administration.

5.3 Study Product Accountability

The study product will be stored in and dispensed by the University of Maryland Medical Center (UMMC) Investigational Drug Service (IDS) pharmacy. The FDA requires accounting for the disposition of all investigational products. The Investigator is responsible for ensuring that an accurate record of product disposition is maintained and product is dispensed only by authorized personnel as required by applicable regulations and guidelines. Records of product disposition as required by federal law consist of the date received, date administered, quantity administered, and the subject number to whom the study product was administered. The investigational pharmacist will be responsible for maintaining accurate records of the shipment and accountability of the study product. The preparation and dispensing of the study product is performed by the IDS Pharmacy and the dosing of subjects is performed by CVD research staff; the process and procedures are documented and maintained in a secure location that is segregated from the blinded research staff. The pharmacy records (both IDS pharmacy and CVD) will be made available for inspection by external monitors and by the relevant regulatory agencies (e.g., FDA) at any time.

5.4 Masking of the Study Product

A prospectively assigned vaccinator and checker will be designated as unblinded personnel and will not have any role in the management of illness or assessment of AEs in study participants. Unblinded personnel will be responsible for the preparation of the study products and for the blinded labeling of the study products. The unblinded staff will be responsible for maintaining the randomization key and will secure these study documents in a locked cabinet.

The PI or the Data and Safety Monitoring Board must submit a written request, to inform each other, for emergency unblinding, if this were to be performed. The written request must clearly state the justification for the emergency unblinding and describe which specific participants are to be unblinded. This document will be filed in the study regulatory file.

5.5 Challenge Agent, *S. flexneri* 2a strain 2457T

GMP master cell banks (MCBs) of *S. flexneri* 2a strain 2457T were produced by the Bioproduction Facility of the Walter Reed Army Institute of Research (WRAIR, Forest Glen, MD). Long-term storage of the challenge inoculum MCB vials is at -80°C or colder.

5.5.1 Preparation of Challenge Inoculum

The challenge inoculum is prepared from a frozen MCB vial, which is plated onto TSA containing Congo red dye (0.01%). After incubation at 35°C for 18-24 hours, single, isolated colonies that exhibit characteristic *Shigella* morphology and that are Congo red positive are to be confirmed as *S. flexneri* 2a using specific monoclonal type II and group 3, 4 *Shigella* antisera. Several well-isolated Congo red colonies will be picked and emulsified in sterile saline. The saline is then used to inoculate, for heavy growth, TSA

plates that are incubated overnight at 35˚C. Overnight growth from the TSA plates is then harvested into sterile phosphate buffered saline (PBS), pH 7.4 and washed 3 times. The heavy *Shigella* suspension is diluted with additional sterile PBS to produce a suspension with an optical density (OD) at 660 nm corresponding to the desired bacterial count per mL—i.e., target dose of 1500 cfu. To determine the actual inoculum of challenge strain ingested, replicate colony counts will be performed pre- and post-challenge. The inoculum will be delivered to the study site on wet ice and used within 4 hours of preparation.

5.5.2 Preparation of Bicarbonate Buffer

Sodium bicarbonate (NaHCO3) is a white, crystalline powder that has a molecular weight of 84.01 gram/Mol and meets USP standards. This product comes in a 500 g plastic container and is stored at room temperature. For each challenge inoculum to be ingested, a buffer solution will be prepared by mixing 2 g sodium bicarbonate with 150 mL nonbacteriostatic sterile water. A 30 mL aliquot of the buffer solution will be set aside for the administration of the challenge agent

5.5.3 Administration of Challenge Agent to Participants

The challenge agent is to be administered orally in a buffer solution. Each subject will drink 120 mL of sodium bicarbonate buffer solution. Approximately 1 to 2 minutes later, the subject will ingest approximately 1500 cfu of *S. flexneri* 2a strain 2457T suspended in 30 mL of the bicarbonate buffer solution. Subjects should have no food or water for 90 minutes pre- and post-inoculation. Subjects will be observed during the 90 minutes after challenge for retention of the challenge inoculum and any immediate AEs/SAEs.

6 STUDY PROCEDURES/EVALUATIONS

6.1 Study Procedures

Informed Consent. All aspects of the protocol will be explained in depth to subjects. Subjects who provide informed, written consent will be screened for eligibility. To evaluate comprehension of the study and to document that informed consent has been obtained, all subjects must pass a written examination before vaccination, *Comprehension Assessment Tool*. The exam contains approximately 20-25 multiple choice and true-false questions on all aspects of the protocol. Staff will review incorrect answers with the subject. A subject who scores below 70% may take the test a second time but will be excluded if a passing score ≥ 70% is not obtained.

Medical Screening. After signing the consent form, verification of Notification of Privacy Practices (NPP) receipt, and HIPAA authorization forms will be completed. Subjects will undergo a clinical evaluation to ensure that they are in good mental and physical health, which will include a medical history, vital signs, and a brief physical examination (oral cavity, auscultation of heart and lungs, abdominal examination, lymph nodes). Blood will be drawn for a complete blood count with differential (WBC, ANC, hemoglobin, platelets), blood chemistries (creatinine, ALT, total bilirubin), serological test for HIV, hepatitis C, hepatitis B surface antigen, serum IgA, HLA-B27 typing, and a *S. flexneri* 2a LPS ELISA titer. Urine will be collected for a urinalysis. A stool sample will be collected for bacterial culture (for *Salmonella*, *Shigella*, *Campylobacter*, *Yersinia*, and *V. cholerae*), prior to challenge. Serum pregnancy test in females of child-bearing potential must be negative during screening and a urine pregnancy test must be negative within 24h of each vaccination. A 12-lead electrocardiogram will be performed.

Subjects who are excluded based on screening results will be counseled by the clinical staff and referred for medical care, as appropriate. If elevated blood pressures are detected after enrollment, the investigator will make a determination whether continued participation would be unsafe or would interfere with the study evaluations.

Blinded Vaccination. The vaccine and placebo will be administered by the IM route by needle and syringe. To maintain the blind, a member of the investigative team who will not be involved in the clinical evaluation of the subjects will be responsible for preparing and labeling the vaccine and placebo with a randomized number. An unblinded checker will be present to verify that the correct randomization scheme has been followed.

Acclimatization for Challenge Phase. Study participants will begin the Challenge phase with admission to the inpatient unit for 2 days of acclimatization, during which time we observe for normal (grade 1-2) stool patterns, we educate and familiarize each subject with the protocol-required procedures (e.g., stool handling), hygiene practices, and the “Rules and Procedures” to be followed while on the inpatient unit. In addition, during the acclimatization period, we monitor behavior, person-to-person interactions, mood, etc. to assess each study participant for any behavior or attitudes which might not be appropriate for an inpatient study (i.e., combativeness, anti-social behavior, anger outbursts, destruction of property, etc.). Any evidence that a subject may demonstrate behaviors which might pose a safety risk to themselves, other subjects, or staff could be cause for ineligibility for challenge (whereupon these subjects are discharged prior to challenge administration). Refusal to comply with protocol-required procedures, adherence to hygiene practices, or repetitive breaking of the “Rules and Procedures” could also constitute ineligibility. This observation during the acclimatization period may be considered an imperfect and rather subjective method, but we have not identified any other good alternate objective

measures that substitute for this direct observation procedure. Any subject who is deemed ineligible will be discharged, prior to challenge.

During the time when a U.S. Public Health Emergency for COVID-19 exists, a SARS-CoV-2 molecular diagnostic test will be performed on each participant upon admission to the inpatient unit. A negative test result and the absence of COVID-19 symptoms will be required for final eligibility for challenge. All subjects must have completed COVID-19 vaccination at least 14 days prior to challenge.

Inpatient Observation. Subsequent to challenge, participants will remain on the ward for approximately 10 days. Vital signs and oral temperature will be measured at least every 8 ±1 hours by staff nurses who remain on the ward 24h a day. All stools will be graded for consistency, loose stools (grade 3 or higher, diarrheal stools) will be weighed and the presence of blood will be confirmed by hemoccult test. Participants will be interviewed daily by a study physician to determine the occurrence of illness signs and symptoms (e.g., anorexia, malaise, abdominal cramps, headache) which occurred during the previous study day; these data will be recorded on a standardized form and graded in severity according to a standardized scale. A focused physical examination may be performed at the discretion of the physician according to the nature of a participant's complaint.

The 5th floor Research Isolation Ward is located on Pharmaron (800 W. Baltimore Street, Baltimore, Maryland) and consists of a total of 48 beds, available as 3 bed dormitory rooms and 12 bed dormitory rooms and which allows for the space to have a flexible configuration for different study size requirements. Each bed is provided with a privacy curtain. There are 10 toilets on the ward. For enteric challenge studies, portable commodes are employed as needed and upon request, to be located adjacent to the bedside. General ward rules and procedures are employed which includes a description of the expectation for maintaining privacy for all participants and for hygiene practices.

Measurement of Diarrhea and Dysentery. Since diarrhea and/or dysentery are key measures of shigellosis, all participants will be expected to collect every stool that is passed, from the time of challenge until discharge. Participants will be instructed to use a plastic stool collection basin, commonly called a "Napolean hat". All stools will be graded by the study staff. The grading of stool is based on consistency and the definition of diarrhea is a grade 3 or higher stool, as follows:

- Grade 1 – well formed (normal stool, does not take the shape of the container)
- Grade 2 – soft (normal stool, does not take the shape of the container)
- Grade 3 – thick liquid (diarrhea, takes the shape of the container readily)
- Grade 4 – opaque watery diarrhea
- Grade 5 – rice water diarrhea (clear watery)

Any grade 3 or higher stool is considered a diarrheal consistency stool and must be weighed, to estimate the volume of fluid loss (assume ~1 g diarrheal stool = 1 mL of fluid lost). Stools must be evaluated with a hemoccult test, for confirmation of dysentery.

Similarly, any episodes of vomiting should be collected in either a stool "hat" or a plastic "kidney" basin, provided. If a vomiting or diarrhea episode is not able to be collected in a basin (e.g., the subject has an "accident" while sleeping or before they are able to reach a toilet), then the volume of the output will be estimated.

Management of Fluid Losses. Subjects who develop diarrheal stools (grade 3 or higher) during the inpatient observation will be required to ingest standard World Health Organization (WHO) Oral Rehydration Solution (ORS) at 1.5 times the stool volume. Vomitus will be replaced with

ORS in equal amounts, 1:1 ratio. At the discretion of the investigator, additional ORS may be administered. If a subject develops severe watery diarrhea or persistent vomiting and cannot maintain full hydration by oral means, IV fluid replacement will be administered.

For the duration of diarrhea, the subject will be requested to provide a urine specimen from every void that they experience; the specimen will be tested for urine specific gravity. In the event that intravenous fluids are required, serum electrolytes (Sodium, Potassium, Chloride, Bicarbonate), BUN, and creatinine will be measured.

A physician is available by telephone or beeper at all times. Nurses will notify the on-call physician if any of the following occurs in a subject who is experiencing diarrhea and/or vomiting:

- Syncope
- Complaint of dizziness or lightheadedness or established orthostatic hypotension
- Urine specific gravity > 1.025
- > 500 mL behind in ORS replacement
- Vomiting of ≥ 500 mL once or total volume within the past 4 hours
- High fever ≥ 39°C (102.2°F)
- Severe headache, severe malaise, or severe abdominal pain
- Subject has a complaint for which he/she requests treatment
- Any other clinical situation that concerns the nurse

Frequency of Vital Signs Assessment. Vital signs (blood pressure, pulse, and oral temperature) will be measured approximately every 8 hours, unless more frequent monitoring is needed. Once a subject has passed a diarrheal stool (grade 3 or higher), vital signs will be measured every ~4 hours until the subject passes a grade 1 or 2 stool or 24 hours have passed since the last grade 3 – 5 stool, whichever comes first. Vital signs will also be measured every ~4 hours when a subject has a fever ≥ 38°C (100.4°F), until the subject has 2 consecutive temperatures of < 38°C (100.4°F). Any subject that complains of dizziness or lightheadedness upon standing will have orthostatic blood pressures assessed—BP after supine for ~5 minutes and BP after standing ~2-3 minutes. Orthostatic hypotension is defined as a drop in systolic BP > 20 mmHg or in diastolic BP > 10 mmHg.

Fluid Therapy/Hydration. Oral Rehydration Solution (ORS) will be offered as the primary means of hydration. ORS will be prepared according to the manufacturer's package insert (Jianas Bros ORS). Unused ORS should be discarded 24 hours after preparation.

Intravenous fluids (Lactated Ringers solution) will be administered to subjects with diarrhea who meet any of the following criteria:

- Orthostatic hypotension
- Urine specific gravity > 1.025, or determined as necessary by the Investigator
- No urine output for ≥ 8 hours
- > 1000 mL deficit in ORS replacement
- At the investigator's discretion, based on clinical evaluation or on a subject's difficulty in keeping up with ongoing diarrheal losses by oral rehydration alone

Intravenous therapy will continue until the above criteria are no longer satisfied, the subject is able to take fluid by mouth, and a study physician determines that it is no longer required.

Indications for Antibiotics. Antimicrobials will be administered immediately if a subject meets one of the primary endpoints in the criteria described under ***Appendix E***. Otherwise, for challenge participants that do not meet criteria for early treatment with antibiotics, they are to initiate

antibiotic treatment 5 days (120 hours) after receiving the challenge. Antibiotics may also be initiated upon investigator judgement, for the safety or welfare of the participant.

Antimicrobials will consist of at least a 3 day course of ciprofloxacin (primary, preferred treatment) or trimethoprim-sulfamethoxazole (secondary, back-up option for treatment). In rare and unexpected instances, the study investigator will be allowed to administer antimicrobials for alternate reasons—e.g., if in their judgment it is necessary to ensure the safety of the subject. Ciprofloxacin dosing will begin with a 1000 mg loading dose followed by 500 mg doses administered every 12 hours (± 1 hr.). Trimethoprim (160 mg)/sulfamethoxazole (800 mg) every 12 hours (± 1 hr) for 3 days will be substituted in the event of an allergy or adverse reaction to ciprofloxacin. Ampicillin 500 mg by mouth every 6 hours (± 1 hr) for 5 days will be administered in the unlikely event that a woman becomes pregnant.

Indications for Other Concomitant Medications. The use of any such PRN medications which are over-the-counter medications will need to be discussed with and approved by the investigator prior to admission. A single prescription medication that has been declared and reviewed upon admission and which has received pre-approval from the investigator (for the medication and the condition it is treating) may be allowable. This prescription medication may be secured by the study team and access will be made available for subject self-dispensing throughout the inpatient stay. Deviations from the prescribed dosage and/or frequency of the medication may be judged by the investigator as grounds for subject non-compliance or may constitute a violation of the ward “Rules and Procedures.” Otherwise, the eligibility criteria forbid the use of two or more chronic concomitant medication use. Any un-declared prescription or over-the-counter medications that are discovered during the inpatient stay will constitute a violation of the ward “Rules and Procedures.”

Other medications may be administered during the study period as follows:

- Smoking is not allowed on the ward, but subjects will be able to request nicotine patches.
- Anti-pyretics and analgesics (i.e., ibuprofen, acetaminophen, aspirin, or similar non-steroidal agents) may be prescribed for severe headaches, other pains, or fevers (e.g., sustained temperatures of ≥ 102.2°F or 39°C).
- At the investigator’s discretion (e.g., upon review of serum electrolyte results during severe diarrhea), oral potassium may be administered for repletion of electrolyte losses.
- Other medications, which are deemed necessary for the safety and welfare of the subject

The prescription of any medication (except the pre-approved single prescription medication mentioned above) must be ordered and signed by the investigator and each administration recorded. Verbal orders are allowable and are to be recorded by the nurse and signed by the ordering physician within 24 hours.

Stool Microbiology. Stool cultures for identification of *Shigella* will be performed to characterize the pattern of fecal shedding. After challenge, all stools will be collected, graded, and weighed while volunteers are on the Research Isolation Ward. Qualitative and quantitative cultures will be performed on the first two stool samples of each 24 hour period. Once antibiotics are initiated, qualitative culture only will be performed. During the outpatient follow up visits within 1 week of discharge (visit 6), 1 month after challenge (visit 7) and last visit (visit 9), stool will be collected for qualitative culture to ensure no presence of *Shigella*; should *Shigella* be identified, then a repeat 3-day course of antibiotics will be required for that participant. If a person is unable to produce a stool by near midnight (a 24 hour period), then a rectal swab will be performed for qualitative culture only. *Note: the exception is the screening stool culture, which is to be evaluated through a CLIA-approved clinical laboratory and is intended to rule out the presence of enteric pathogens.*

In place of the screening stool culture, as performed by standard bacteriologic culture methods, it is also acceptable to use a CLIA-approved molecular diagnostic test to rule out the presence of enteric (e.g., BioFire FilmArray GI Panel).

Shigella Disease Severity Scoring. Using clinical data that is planned to be collected by the investigator during the challenge phase, an ordinal disease scoring system will be used to characterize the disease elicited by the challenge model, using a previously published method.[44] The scoring of disease severity consists of several parameters, including objective signs, subjective symptoms, and loose stool (grade 3 or higher) output over 24 hours. The signs, symptoms, and loose stools from the time of challenge and through 24 hours after the initiation of antibiotics will be used in the *Shigella* Disease Severity Scoring system.

For objective signs, each of the following adds one point to the score: Gross blood in ≥2 loose stools (hemoccult confirmed), maximum temperature >101.1°F, any vomiting.

Subjective symptoms are based on the severity (severe: prevents routine activity; moderate: some interference with routine activities; mild: does not interfere with routine activities) of arthralgia, nausea, myalgia, headache, anorexia, abdominal cramps/pain. If more than one of those subjective symptoms is coded as severe the subject will have a subjective symptom score of 3, if only one is coded as severe, their score is a 2, and if the subject has multiple moderate symptoms (but none is coded as severe), their score is a 1. If the subject has only one moderate (or only mild or no symptoms), it is 0. Loose stool output is coded as follows based on the maximum 24 hour output: score of 0 for ≤1 loose stool in 24 hrs, score of 1 for 2-3 loose stools in 24 hrs, score of 2 for 4-8 loose stools in 24 hrs; and score of 3 for ≥9 loose stools in 24 hrs. Total *Shigella* Disease Severity Scores may range from 0-9.

All the elements which contribute to the *Shigella* Disease Severity Score are obtained from data that is collected by the study team (i.e., there will not be additional subject self-reporting through questionnaires to collect these data); see ***Appendix F*** for the collection of subjective symptoms.

***Shigella* Disease Severity Scoring**		
Parameter	**Outcome**	**Score**
Objective signs	Gross blood in ≥2 loose stools (hemoccult confirmed)	1
	Maximum temperature (°F); >101.1	1
	Vomiting	1
Subjective symptoms	More than one of the following as severe: arthralgia, nausea, myalgia, headache, anorexia, abd cramps/pain	3
	Any one of the following as severe: arthralgia, nausea, myalgia, headache, anorexia, abd cramps/pain	2
	More than one of the following as moderate: arthralgia, nausea, myalgia, headache, anorexia, abd cramps/pain	1
Loose stool output (max 24 hr freq)	0-1	0
	2-3	1
	4-8	2
	≥9	3

6.2 Laboratory Evaluations

6.2.1 Laboratory Evaluations/Assays

Screening Clinical Laboratory. The following clinical laboratory assessments will be performed as part of the screening for eligibility (***Appendix B***):

- CBC with differential for total white blood cell count (WBC), absolute neutrophil count (ANC), hemoglobin (Hg), platelet count
- Creatinine, alanine aminotransferase (ALT), total bilirubin
- Serum IgA
- Hepatitis B surface antigen, Hepatitis C virus antibody, HIV
- β-HCG (if woman of child-bearing potential)
- HLA-B27 histocompatibility testing
- Urinalysis for glucose, protein, and occult blood
- Stool culture or BioFire FilmArray Gastrointestinal (GI) Panel (or equivalent CLIA-approved molecular diagnostic test) – eligibility for challenge not vaccination

For clinical laboratories to be evaluated, the following tubes may be used:

- *4 mL EDTA for CBC with differential*
- *8.5 mL tiger top for chemistries, serum IgA, HBsAg, HCV, β-HCG*
- *4 mL EDTA for HLA-B27 test (Monday through Thursday shipments only)*
- *3.5 mL tiger top for HIV test*

Screening *S. flexneri* 2a LPS IgG titer. Approximately 5 mL of whole blood will be collected for serum. The standardized serum *S. flexneri* 2a LPS IgG ELISA will be performed, as per the previously published methodology.[45] Screening titers are calculated as the inverse of the serum dilution. Subjects with screening titers of >2500 will be excluded from the study.

12-lead Electrocardiogram (ECG). A standard 12-lead ECG will be performed as part of screening for eligibility. Pathological abnormalities will be exclusion criteria, as described in ***Appendix B***.

Safety Laboratory. The following clinical laboratory assessments will be performed at the Day 36 visit (grading of toxicity is according to ***Appendix C***):

- CBC with differential for total white blood cell count (WBC), absolute neutrophil count (ANC), hemoglobin (Hg), platelet count
- Creatinine, alanine aminotransferase (ALT), total bilirubin

Stool for Culture During Acclimatization. A stool culture will be conducted from a stool specimen provided during the acclimatization. The results may not need to be available for challenge and are intended to definitively document the absence of enteropathogens prior to challenge.

6.2.2 Special Assays or Procedures

ELISA Antibody in Serum, Urine, and Stool. Serum, urine, and stool antibody responses will be measured against *S. flexneri* 2a LPS. Standardized ELISAs for immunoglobulin G (IgG) and immunoglobulin A (IgA) will be used to quantify antibody responses.[33,46-48]

ELISA titers will be calculated as the inverse serum dilution that produces an absorbance value (405 nm) of 0.2 above background. ELISA for serum and urine antibodies to *S. flexneri* 2a LPS will be performed at Tel Aviv University (Prof. Dani Cohen). ELISA for serum antibodies to *S. flexneri* 2a LPS will be performed at WRAIR (Dr. Robert Kaminki) and CVD (Dr. Marcela Pasetti).

Serum Bactericidal Antibody (SBA). The functional activity of immune sera will be analyzed using the serum bactericidal antibody (SBA).[49] SBA titers are calculated from the reciprocal of the serum dilution that produces 50% bacterial killing. The SBA tests will be performed at Tel Aviv University (Prof. Dani Cohen).

Antibody-Secreting Cells (ASC). Plasmablasts secreting antibodies that recognize *S. flexneri* 2a LPS will be measured using ELISPOT.[33,46] The frequency of spot-forming cells (SFC) from replicate wells will be determined and reported as ASC/10^6 PBMC. A positive response will be defined as ≥8 ASC/10^6 PBMC.

Antibodies in Lymphocyte Supernatants (ALS). ALS supernatant fluid will be collected and tested in an ELISA assay to measure antigen-specific IgA and IgG antibodies released by the PBMC.[50] Endpoint titers will be calculated as the reciprocal dilution giving rise to an absorbance value of 0.4 above the background at 450 nm. A positive response will be defined as a ≥4-fold increase in ALS titer over baseline.

ASC Homing Studies. To measure the homing potential of plasmablasts, PBMC will be enumerated by flow cytometry based on molecules expressed in naïve (B_n; CD19+ IgD+ CD27-) and memory B (B_M; CD19+ IgD- CD27+) cells which direct them to home to distinct sites (e.g., integrin α4β7, CD62L, CXCR3).[51] Several of these populations (e.g., B_n, B_M expressing combinations of CXCR3, integrin α4β7 and/or CD62L] will be sorted and their ability to secrete specific anti-*S. flexneri* LPS antibodies will be determined by ELISPOT as described for ASC determinations. Sorting will be performed using a MoFlow Astrios EQ flow cytometer/cell sorter state-of-the-art system available in the CVD Flow Cytometry and Mass Cytometry Core Laboratory.

Qualitative/Quantitative Stool Culture. For each stool specimen, a sample of the "whole stool," will be placed in a sterile specimen container and then kept for short-term storage at 2-8°C. A sterile cotton swab (or the rectal swab) will be inserted into the stool (sampling nearest the most loose or liquid parts of stool or areas with gross blood) and then placed into a tube containing BGS transport medium for short-term storage at 2-8°C. The CVD Microbiology Lab will plate the "whole stool" specimen for quantitative culture of *S. flexneri* 2a strain, and will plate the stool in BGS for qualitative culture of S. *flexneri* 2a strain. S. *flexneri* 2a strain will be confirmed by agglutination with commercial typing antisera.

Stool Cytokine Analysis. For each day post-challenge (days 57-64), a single representative stool from each challenge participant will be used to assess calprotectin and myeloperoxidase (MPO). Whole stools will be collected and placed into sample collection containers, as provided by the commercially available kits to be purchased from Epitope Diagnostics, Inc. (San Diego, CA): KT-849 for quantitative fecal calprotectin ELISA kit and KT-844 for the fecal MPO sample collection kit. This assay will be performed at WRAIR, Department of Subunit Enteric Vaccines & Immunology (Dr. Robert W. Kaminiski).

Future Studies. Stool for microbiome (e.g., 16S rRNA profiling) and transcriptomics, blood transcriptomics, serum microarray, or other analyses which would be for the

characterization of responses to vaccination or challenge may be performed in the future on banked specimen.

6.2.3 Specimen Collection, Preparation, Handling and Shipping

6.2.3.1 Instructions for Specimen Preparation, Handling, and Storage

Instructions for specimen preparation, handling, and storage are described in the ***Manual of Procedures*** (***MOP***).

6.2.3.2 Specimen Shipment

Clinical laboratory specimens will be shipped to collaborators at Tel Aviv University (Israel), Walter Reed Army Institute of Research (Silver Spring, MD), and Institut Pasteur (Paris, France) for immunological analysis as outlined in the ***MOP***. Research study specimens are intended to be stored until complete use for future-use research.

7 STUDY SCHEDULE

Complete study schedule details listed by type of visit are described below. Refer also to ***Appendix A: Study Schedule of Events***.

7.1 Screening (-60 to -1 days prior to enrollment)

Potential volunteers may be screened for eligibility up to 60 days prior to enrollment. Interested potential participants, responding to advertisements, campus flyers, or notification through existing databases, will be instructed to call the CVD to receive further information about the study. CVD research staff receives these inquiries and read through an IRB-approved telephone script which describes basic information on the study topic, duration and number of visits, procedures, and eligibility criteria. No personal health information is solicited and afterwards interested potential participants can be given an appointment to attend an *Orientation Session* for formal screening.

At the *Orientation Session*, the consent process continues. CVD research staff will provide a detailed description of all aspects of the study, including the rationale and background, the public health significance, the procedures and schedule of visits, and a detailed discussion of the risks and the presence or absence of benefit to them, as appropriate. This *Orientation Session* is commonly one hour long. The prospective subject is given a copy the study consent form (without the signature page) and is encouraged to discuss his/her participation with family members or advisors before agreeing to sign the informed consent form and proceeding with any screening procedures.

After the informed consent form is signed, the subject is interviewed one-on-one by a member of the study team to discuss the study. A brief written examination is administered to assess the volunteer's comprehension of the study (i.e., *Comprehension Assessment Tool*). If this quiz is passed (≥70% correct answers), the research staff complete the medical history and concomitant medication forms, draw blood for eligibility testing, and obtain an ECG. Study subjects are also asked to sign a verification of Notification of Privacy Practices receipt and Health Insurance Portability and Accountability Act (HIPAA) authorization form. The subject also meets the PI, or designee, and has a physical examination to complete the eligibility screening. If the subject has passed all the eligibility criteria, he/she is then invited to proceed in the study and is given an appointment for the next visit. The screening procedures may be completed in a single day or on multiple days, as long as the screening procedure dates do not exceed 60 days prior to vaccination.

It is recognized that volunteer studies must be carried out in an environment where no coercion is applied, and where volunteers can be adequately informed of the purpose, nature, procedures, risks and hazards of the study. To assess and document comprehension of the material presented, each subject must pass a written quiz (score 70% or higher) containing approximately 20 multiple choice and true/false questions covering all aspects of the study including the purpose, procedures, risks, benefits and pertinent microbiology. Incorrect answers will be reviewed with the subject. The quiz may be retaken once, after a review of the consent form. The *Comprehension Assessment Tool* is dated and signed by the subject and by a research staff member and made part of the permanent record.

Another important feature of our consent process is the repeated demonstration of both initiative and reliability by the prospective subject. There are multiple opportunities for the subject to decline to proceed further in the process. This deliberate education and screening process contributes to the informed nature of the subject's consent. The process also increases the likelihood that the subject will be committed to completing the entire inpatient containment portion of this study. Prospective subjects will be carefully screened to ensure that they are in good physical and mental health.

The screening procedures include:

- Signed, witnessed informed consent
- Administration of the study Comprehension Assessment Tool
- Obtaining vital signs (oral temperature, blood pressure, pulse, height, and weight)
- Collection of medical history
- Collection of concomitant medication history
- Perform physical examination, to be performed by a study clinician
- Perform 12-lead electrocardiogram (ECG); the interpretation of screening ECG eligibility is defined in ***Appendix B***.
- Obtain the following screening laboratory studies (~25 mL blood); the acceptable values for the screening laboratory tests are defined in ***Appendix B***:
    - Complete Blood Count (CBC) with differential and platelet count for the evaluation of WBC, ANC, Hemoglobin, and Platelets
    - Creatinine, ALT (SGPT), and Total Bilirubin
    - Serum IgA
    - Serum Sf2a LPS IgG
    - HLA-B27 histocompatibility
    - Urinalysis for the evaluation of glucose, protein, and blood
    - Pregnancy test (if female of child-bearing potential, serum β-HCG)
    - HIV antibody
    - Hepatitis B surface antigen
    - Hepatitis C antibody

Note: Since intravenous fluids is a part of the planned management of severe diarrhea, if more than 3 attempts at venipuncture are required for obtaining screening labs, then we will consider that person in eligible on the basis of poor venous access.

7.2 Visit 1 - Enrollment and Vaccination Dose #1 (Day 1)

Study volunteers that have been determined to be eligible for participation through screening will be scheduled for enrollment. Upon arrival to the outpatient research clinic, each study participant will have the following procedures performed:

- Confirmation of ongoing consent
- Review of changes to medical history, concomitant medications, travel history
- Vital signs assessment (oral temp, blood pressure, pulse)
- Physical examination, if indicated
- Review and affirmation of eligibility criteria
- For women of childbearing potential, urine pregnancy test
- For Cohorts 1-3:
    - Collection of stool sample, at least 5 grams
    - Collection of urine sample, at least 20 mL

- Collection of venous blood sample, 15 mL in serum separator tubes and 110 mL in EDTA vacutainers

Enrollment and randomization may be performed after eligibility has been confirmed. Once randomization has been completed, the participant will receive a single dose of blinded study product per randomized assignment via IM injection in the deltoid muscle of the preferred arm.

- After vaccination, subjects will be observed in the clinic for at least 20 minutes. The vaccination site will be examined, and any AE/SAEs will be assessed prior to discharge from the clinic.
- Subjects will be provided with a Memory Aid document and will be instructed on how to use the memory aid and how to measure and record AE/SAEs prior to discharge from the clinic.
- A digital thermometer will be provided for the assessment of daily oral temperatures; subjects will be encouraged to take their temperature around the same time each day.
- Subjects will be instructed to notify the study center if they develop any severe reactions following vaccination

7.3 Visit 2 – Clinic Follow-up Post-Dose #1 (Day 8, +1 day window)

- Confirmation of ongoing consent
- Vital signs assessment (oral temp, blood pressure, pulse)
- Physical examination, if indicated
- Collect and review the information on the Memory Aid document
- Review for any unsolicited AEs and concomitant medications
- For Cohorts 1-3:
  - For participants in the Homing Studies Subset, collection of venous blood sample, 15 mL in serum separator tubes and 50 mL in EDTA vacutainers
  - For those not in the homing subset, collection of venous blood sample, 15 mL in serum separator tubes and 20 mL in EDTA vacutainers
- Provide stool collection kit and instructions for next clinic visit

7.4 Visit 3 – Vaccination Dose #2 (Day 29, ±2 day window)

- Confirmation of ongoing consent
- Review of changes to medical history, concomitant medications, travel history
- Vital signs assessment (oral temp, blood pressure, pulse)
- Physical examination, if indicated
- Review and affirmation of eligibility criteria
- For women of childbearing potential, urine pregnancy test
- For Cohorts 1-3:
  - Collection of stool sample, at least 5 grams
  - Collection of urine sample, at least 20 mL
  - Collection of venous blood sample, 15 mL in serum separator tubes and 110 mL in EDTA vacutainers

Once confirmation of eligibility of vaccination has been completed, the participant will receive a single dose of blinded study product per randomized assignment via IM injection in the deltoid muscle of the preferred arm.

- After vaccination, subjects will be observed in the clinic for at least 20 minutes. The vaccination site will be examined, and any AE/SAEs will be assessed prior to discharge from the clinic.
- Subjects will be provided with a Memory Aid document and instructed on how to record AE/SAEs

7.5 Visit 4 – Clinic Follow-up Post-Dose #2 (7 days post-2nd vaccination, +1 day window)

The timing of this visit is intended to be approximately Day 36 but is intended to be flexible because of the dependence of this visit according to the receipt of the second dose of vaccine.

- Confirmation of ongoing consent
- Vital signs assessment (oral temp, blood pressure, pulse)
- Physical examination, if indicated
- Collect and review the information on the Memory Aid document
- Review for any unsolicited AEs and concomitant medications
- Collection of clinical safety laboratory, 4 mL in purple top tube and 3.5 mL in red top tube
- For Cohort 1-3:
    - For participants in the Homing Studies Subset, collection of venous blood sample, 15 mL in serum separator tubes and 50 mL in EDTA vacutainers
    - For those not in the homing subset, collection of venous blood sample, 15 mL in serum separator tubes and 20 mL in EDTA vacutainers
- Collection of a stool sample for stool culture or BioFire FilmArray Gastrointestinal (GI) Panel (or equivalent CLIA-approved molecular diagnostic test). *This is being performed as a screening procedure prior to challenge. This procedure is allowed to be collected with a +21 day window*.

7.6 Inpatient Containment Period (~Day 55-66, or challenge timed to be ~28 days post-2nd vaccination)

Study participants that have received two doses of blinded study product and are available for the challenge phase may be scheduled for admission to the inpatient containment unit (research isolation ward). The timing of the inpatient and challenge is intended to be approximately 28 days (+7 days window) from the completion of the second dose of vaccine. The anticipated duration of the inpatient stay is 12 days.

7.6.1 Acclimatization (2 days prior to challenge)

Study participants will start their inpatient stay with 2 days for acclimatization, during which we educate and familiarize each subject with the protocol-required procedures (e.g., stool handling), hygiene practices, and the “Rules and Procedures” to be followed while on the inpatient unit.

An important aspect of the acclimatization period is the performance of a psychological evaluation which is intended to reduce the risk of problems with subject compliance and potential risks to the safety and welfare of participants. In the past, a contracted

psychiatrist performed a formal psychological evaluation of mental health on each participant prior to challenge. This approach was expensive, time-consuming, and was not entirely adequate for predicting the appropriateness of an individual for an inpatient stay. Therefore, the approach, which is currently in use, is an informal psychological evaluation. During the 2-day acclimatization period, we closely monitor behavior, person-to-person interactions, mood, etc. to assess each study participant for any behavior or attitudes that might not be appropriate for an inpatient containment study (i.e., combativeness, anti-social behavior, anger outbursts, destruction of property, etc.). Any evidence that a subject may demonstrate which might pose a safety risk to themselves, other subjects, or staff could be cause for ineligibility for challenge and the remainder of the inpatient stay. Refusal to comply with protocol-required procedures, adherence to hygiene practices, or repetitive breaking of the "Rules and Procedures" could also constitute ineligibility through our informal psychological evaluation. This informal psychological evaluation during the acclimatization period may be considered an imperfect and rather subjective method, but we have not identified any other good alternate objective measures that substitute for this direct observation procedure. The psychiatrist-generated formal psychological evaluations were not a better predictive tool than the direct observation approach. Nonetheless, all participants will be provided the opportunity to discuss with a psychologist before entering, during inpatient containment, and after discharge if he/she wishes. Any subject who is deemed ineligible will be discharged, prior to challenge, and back-up eligible participants will be used.

At any time during acclimatization (prior to ingestion of challenge), the following will be collected:

- Stool specimen, for stool culture, fecal IgA, transcriptomic, microbiome
- Urine specimen, at least 20 mL
- Venous blood specimen, 15 mL in serum separator tubes, and 110 mL in EDTA vacutainers
- During the period when a U.S. Public Health Emergency exists, a SARS-CoV-2 molecular diagnostic test will be performed, which must be a negative test result prior to challenge

7.6.2 Challenge

On the morning of challenge, subjects will have baseline vitals (oral temperature, pulse, and blood pressure) recorded and a final eligibility confirmation will be completed prior to oral ingestion of the challenge inoculum.

After confirmation of at least 90 minutes of fasting, eligible subjects will drink 120 mL of sodium bicarbonate buffer solution (~1.3% $NaHCO_3$); approximately 1-2 minutes later, subjects will ingest the challenge inoculum consisting of ~1500 cfu of strain 2457T suspended in 30 mL of sodium bicarbonate buffer solution. Subjects will have nothing by mouth, for 90 minutes before and after ingestion of the blinded study product. For the 90 minutes after challenge, participants will be observed for any immediate AEs.

All back-up study participants that are not challenged will be discharged from the inpatient ward upon the completion of the target number of challenge recipients.

7.6.3 Post-Challenge Observation Period (until discharge)

For the remainder of the inpatient stay, study participants will be observed on the Research Isolation Ward and the following procedures will be performed:

- All stools will be graded for consistency (grade 1-5) and any diarrheal stool (grade 3 or higher) will be weighed. We assume a 1:1 weight per volume conversion for diarrheal stools. Blood from each stool specimen will be confirmed to have blood with a hemoccult test. Up to two stool specimens per day will be submitted for culture.
- Any vomitus will also be weighed.
- Vital signs will be measured at least 3 times daily (approximately every 8 hours).
- For each day, the maximum of an adverse symptom over that day will be assessed. The solicited adverse symptoms include: nausea, abdominal pain, abdominal cramping, myalgia, arthralgia, malaise, tenesmus, anorexia, and headache.
- For each day, a daily interview will be conducted by the investigator; targeted physical examinations will be performed if necessary.
- For 8 days, 2.5 mL of venous blood into a PaxGene tube
- For participants in the Homing Studies Subset, on day 7 post-challenge, collection of 15 mL of blood into serum separator tubes and 50 mL into EDTA vacutainers
- For those not in the homing subset, on day 3 post-challenge, collection of 30 mL of venous blood into EDTA vacutainers
- For those not in the homing subset, on day 7 post-challenge, collection of 15 mL of blood into serum separator tubes and 50 mL into EDTA vacutainers

The management of clinical illness is described in Section 5.6. For participants that did not satisfy criteria for early treatment with antibiotics (Section 5.6), antibiotic therapy will commence at approximately 120 hours post-challenge.

Meanwhile, challenge participants will be assigned their own bed and dresser and provided all their meals, including breakfast, lunch, dinner, and two snacks per day. During the inpatient stay, there are various forms of entertainment which can be enjoyed, including one large screen television and multiple smaller televisions, a pool table, board games, video games (Xbox and Playstation), WiFi internet access, and two community computers. Laundry is provided with free, self-service washer and dryer machines. Participants are encouraged to bring their own reading material (books and magazines) and laptop computers. The only firm prohibition for materials brought with participant into the inpatient unit are regarding potential weapons, tobacco, or any drugs of abuse or any material which could be offensive (e.g., pornographic material).

7.6.4 Criteria for Discharge

All four of the following criteria below must be satisfied in order to be discharged from the Research Isolation Ward:

- two sequential *Shigella* negative stool cultures, separated by 12 hours
- absence of fever for 24 hours
- absence of diarrhea or dysentery or other GI symptoms for 24 hours
- completed a 3-day course of antibiotics, per protocol

Subjects will receive written instructions to adhere to the following hygienic precautions for 4 weeks after discharge: to wash their hands with soap and water carefully and dry

them completely with a towel each time they use the bathroom, and to avoid sharing their towel with others. Subjects will also be provided a stool collection kit for bringing a stool sample to the scheduled clinic visit #6.

7.7 Visit 5 – Clinic Follow-Up, only for those not in Challenge phase (28 days post-2nd vaccination, ±3 days window)

The timing of this visit is intended to be approximately Day 57 but is intended to be flexible because of the dependence of this visit according to the receipt of the second dose of vaccine.

- Confirmation of ongoing consent
- Vital signs assessment (oral temp, blood pressure, pulse)
- Physical examination, if indicatedl
- Review for any AEs/SAEs and record any concomitant medications
- For Cohorts 1-3:
    - Collection of stool sample, at least 5 grams
    - Collection of urine sample, at least 20 mL
    - Collection of venous blood sample, 15 mL in serum separator tubes, and 90 mL in EDTA vacutainers
    - Provide stool collection kit
- Provide instructions for next clinic visit

7.8 Visit 6 – Clinic Follow-up (~7 days from discharge, +7 day window, only for those in the Challenge phase)

- Confirmation of ongoing consent
- Collection of stool sample, at least 5 grams
- Review for any AEs or SAEs
- Provide stool collection kit
- Provide instructions for next clinic visit

7.9 Visit 7 – Clinic Follow-Up (~56 days post-2nd vaccination, ±3 days window)

The timing of this visit is intended to be approximately Day 85 but is intended to be flexible because of the dependence of this visit according to the receipt of the second dose of vaccine.

- Confirmation of ongoing consent
- Vital signs assessment (oral temp, blood pressure, pulse), if indicated
- Physical examination, if indicated
- Review for any SAEs
- For Cohorts 1-3:
    - Collection of stool sample, at least 5 grams
    - Collection of urine sample, at least 20 mL

  - Collection of venous blood sample, 2.5 mL in PaxGene tube (challenge participants only), 15 mL in serum separator tubes, and 90 mL in EDTA vacutainers
  - Provide stool collection kit
- Provide instructions for next clinic visit

7.10 Visit 8 – Clinic Follow-Up (~16 weeks post-2nd vaccination, ±7 days window)

The timing of this visit is intended to be approximately Day 141 (or 16-weeks after the 2nd dose of vaccine) but is intended to be flexible because of the dependence of this visit according to the receipt of the second dose of vaccine.

- Confirmation of ongoing consent
- Vital signs assessment (oral temp, blood pressure, pulse), if indicated
- Physical examination, if indicated
- Review for any SAEs
- For Cohort 1-3:
  - Collection of stool sample, at least 5 grams
  - Collection of urine sample, at least 20 mL
  - Collection of venous blood sample, 15 mL in serum separator tubes and 90 mL in EDTA vacutainers
  - Provide stool collection kit
- Provide instructions for next clinic visit

7.11 Visit 9 – Last Follow-up (~6 months post-challenge or post-visit 5, ±14 days window)

The timing of this visit is intended to be approximately Day 237 (or 6-months from visit 5 or challenge) but is intended to be flexible because of the dependence of this visit according to the visit 5 challenge date.

- Vital signs assessment (oral temp, blood pressure, pulse)
- Physical examination, if indicated
- Review for any SAEs
- For Cohorts 1-3:
  - Collection of stool sample, at least 5 grams
  - Collection of urine sample, at least 20 mL
  - Collection of venous blood sample, 15 mL in serum separator tubes and 90 mL in EDTA vacutainers

7.12 Unscheduled Visit(s) or Early Termination Visits, if applicable

Subjects that experience any AEs, SAEs, or experience an event of concern can be scheduled for an outpatient visit to be further evaluated. If an unscheduled visit occurs, a member of the clinical study team (PI, subinvestigator, nurse coordinator, or clinical nurse) will interview and

evaluate the subject to determine the cause of the visit and provide care as needed. If an early termination visit were to occur, this visit will be conducted as described in Section 4.4 and ***Appendix A***.

7.13 Special Consideration for COVID-19

While there is a U.S. Public Health Emergency for COVID-19, prior to each scheduled clinic visit the study participants will be requested to self-report any illness symptoms prior to arrival at the clinic so that the study staff may discuss whether the clinic visit should be postponed or cancelled. These procedures should be consistent with the UMB campus research policies regarding COVID-19 practices.

8 SAFETY ASSESSMENT AND REPORTING

The assessment of the safety of the vaccine will be through the detection and documentation of adverse effects, both solicited AEs and unsolicited AEs, and/or clinically significant laboratory abnormalities, from enrollment through 28 days post-vaccination. Only the occurrence of SAEs will be reported between Day 57 (or 28 days post-last dose of vaccine) through the end of the study (Day 237).

8.1 Definition of Adverse Event (AE)

An adverse event is any untoward medical occurrence in a participant after administration of the investigational product and that does not necessarily have a causal relationship with the investigational product. An AE can therefore be any unfavorable and unintended sign (including abnormal laboratory findings), symptoms, physical examinations, or disease temporally associated with the use of the investigational product, whether or not related to the investigational product. This definition includes exacerbations of pre-existing conditions. Stable pre-existing conditions which do not change in nature or severity during the study are not considered AEs; however these should be reported as part of the medical history.

Solicited AEs are pre-specified AEs that could potentially be in association with the blinded study product. There will be no assigning of causality for solicited AEs to the study product or an alternate etiology.

Unsolicited AEs are any AEs reported spontaneously by the participant, observed by the study personnel during study visits or those identified during review of medical records or source documents. Investigators will attempt to assign causality of unsolicited AEs to either the study product or an alternate etiology.

8.1.1 Grading of Severity of an AE

All AEs will be assessed by the clinician using the following guidelines to quantify severity:

- Mild: events require minimal or no treatment and do not interfere with the participant's daily activities.
- Moderate: events result in a low level of inconvenience or concern with the therapeutic measures. Moderate events may cause some interference with functioning.
- Severe: events interrupt a participant's usual daily activity and may require systemic drug therapy or other treatment. Severe events are usually incapacitating.
- Life threatening: any adverse drug experience that places the participant, in the view of the investigator, at immediate risk of death from the reaction as it occurred, i.e., it does not include a reaction that had it occurred in a more severe form, might have caused death.

AEs characterized as intermittent require documentation of onset and duration of each episode.

Laboratory abnormalities will be assessed by the clinician using a protocol-defined grading system (***Appendix C***).

8.1.2 Relationship to Study Product

Relationship (causality or attribution) of all AEs to the study product or to an alternate etiology (unrelated to the study) is part of the documentation process, but it is not a factor in determining what is (or is not) reported in the study. If there is any doubt as to whether a clinical observation is an AE, the event should be reported. All AEs must have their relationship assessed using one of two terms: related to study product or not related (an alternate etiology can be identified and is likely or feasible). To help assess, the following guidelines are used.

- Related to study product (i.e., SF2a-TT15 or placebo) – There is a reasonable possibility (perhaps due to the timing of onset of the symptoms) that the study product caused the AE. Reasonable possibility means that there is evidence to suggest a causal relationship between the study product and the AE, and there is no reasonable alternate etiology.
- Not Related – The event is not related to the study product (i.e., SF2a-TT15 or placebo) because of a reasonable alternate etiology.

8.2 Definition of Serious Adverse Event (SAE)

A serious adverse event (SAE) is any AE that results in any of the following outcomes:

1. Death
2. A life-threatening event. Life-threatening events mean that the study participant was, in the opinion of the site PI or Sponsor, at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe.
3. Requires inpatient hospitalization or prolongation of existing hospitalization; scheduled elective procedures will not be considered an SAE
4. Results in persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
5. Congenital abnormality or birth defect
6. Important medical event that may not result in one of the above outcomes but may jeopardize the health of the study participant and/or requires medical or surgical intervention to prevent one of the outcomes listed in the above definition of serious adverse event. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

8.3 Reporting Procedures

Via memory aid, solicited reactogenicity events will be documented and reported from the time of study vaccination through 7 days after each dose of vaccine (Day 8 and Day 36, respectively). Post-vaccination reactogenicity will include daily: temperature; local signs (pain, redness, induration); and systemic symptoms (headache, fatigue, arthralgia, myalgia, diarrhea, anorexia, chills, and vomiting).

Clinical safety laboratory AEs will be documented and reported.

Unsolicited non-serious AEs will be documented and reported from the time of study vaccination through approximately 28 days after vaccination. Concomitant medications will be recorded and include all current medications and medications taken in the 28 days prior to enrollment and through 28 days after last vaccination (or early termination, if this occurs first). Concomitant medications include prescription and over-the-counter drugs, including herbals, vitamins, and supplements. The receipt of licensed vaccines during this reporting period will also be recorded as a concomitant medication. The use of a new medication may prompt the evaluation of the occurrence of an SAE or a new diagnosis of a chronic medical condition.

SAEs will be documented and reported from the time of the study vaccination through end of study (Day 237, approximately 8 months from enrollment).

8.3.1 Serious Adverse Event Detection and Reporting

All SAEs will be:

- recorded on a Data Collection Form
- reported to the local IRB, per local IRB guidelines
- reported to the Data and Safety Monitoring Board
- reviewed and evaluated by a study clinician and the PI
- followed through resolution by a study clinician

All deaths and immediately life-threatening events, whether related or unrelated, will be reported to both the study sponsor and the DSMB within 24 hours of site awareness. Other SAEs regardless of relationship, will be reported to the study sponsor and the DSMB within 72 hours of site awareness

8.3.2 Reporting of Pregnancy

Pregnancies occurring in study subjects will be reported on the Pregnancy Report form; pregnancies will be reported to the DSMB. No further study vaccinations and/or challenges will be administered to pregnant subjects, but with the subject's permission all protocol-required venous blood samples will be obtained and the subject will continue to be followed for safety for the duration of this trial. Stool and urine specimens will continue to be collected, if the participant agrees. Efforts will be made to follow all pregnancies reported during the course of this trial to pregnancy outcome pending the subject's permission.

All unexpected fatal or life-threatening suspected adverse reaction reports will be submitted to the FDA within 7 days of notification from the site, in accordance with 21 CFR 312.32.

8.4 Halting Rules

Further enrollments, study vaccinations, and challenges will be halted for an ad hoc DSMB review if any of the following events occur:

- One or more participants experience a SAE, that is related to study product
- Two or more subjects with the same severe (Grade 3 or higher) AE following exposure to the study product considered to be related to vaccination

The study will not continue until the DSMB has made the determination that the halt may be lifted. The lifting of a halt may require changes to the protocol and/or informed consent form and is upon the advisement of the DSMB.

8.5 Safety Oversight

An independent DSMB will perform the oversight of safety for this study. The DSMB will consist of up to 5 scientists that are not involved with the conduct of the study. The primary responsibility of the DSMB is to monitor participant safety. The DSMB considers study-specific data as well as relevant background information about the disease, test agent, and target population under study. We plan for the DSMB to review the cumulative safety data shortly after completion of each inpatient phase. The DSMB will be empowered to suspend the study, recommend amendments to the protocol, and/or to request further information for their review. One of the five scientists will be designated as the Independent Safety Monitor (ISM); this person is to be local to the CVD but not a part of the study team. The ISM will be responsible for conducting a comprehensive review, should there be a halt in the study or at the request of the DSMB. Should there be a halt in the study, no further study product dosing or challenges will be performed until the halt is lifted; all enrolled participants will continue to be followed for safety. For participants that are in the Research Isolation Ward at the time of a halt, the management of diarrhea or other symptoms will continue, until resolution. An DSMB Charter will be reviewed and approved by the DSMB members prior to the initiation of the trial and will include the scheduled frequency/timing of DSMB meetings, types of data for review, halting rules, and roles/responsibilities.

9 CLINICAL MONITORING STRUCTURE

Site monitoring is conducted to ensure that the rights and well-being of human subjects are protected, that the reported trial data are accurate, complete, and verifiable, and that the conduct of the trial is in compliance with the currently IRB-approved protocol/amendment(s), with GCP, and with applicable regulatory requirement(s). Monitoring refers to the methods used by sponsors of investigational studies, or Contract Research Organizations (CROs) delegated site monitoring responsibilities, to oversee the conduct of, and reporting of data from, clinical investigations. Site monitoring includes ensuring appropriate clinical investigator supervision of study site staff and third party contractors.

In order to ensure protocol compliance, monitoring visits by a sponsor-designated professional or monitor will occur at scheduled intervals. The visit frequency will be defined in a monitoring plan (refer to Section 8.1 below) and communicated before study start to the PI and all other appropriate study personnel.

9.1 Site Monitoring Plan

Independent site monitoring will be conducted to assess the progress of the clinical trial and ensure that the rights, safety, and well-being of study participants are protected, study procedures, laboratory procedures, study intervention administration, and data collection processes are of high quality that comply with ICH/GCP, federal, state, and local regulatory authorities and that the study is conducted in accordance with the IRB-protocol and sponsor standard operating procedures (SOPs). Clinical monitoring of this protocol will be performed by qualified individuals selected by the sponsor in accordance with ICH/GCP (Section 5.18.2) and as detailed in the monitoring plan.

Site visits will be made at standard intervals as defined by the sponsor. Monitoring visits will performed in accordance with ICH/GCP (Section 5.18) and will include, but are not limited to, review of regulatory files, participant study charts inclusive of Data Collection Forms (DCFs) and source documents contained therein, informed consent forms, study product accountability records, medical and laboratory reports, etc. Study monitors will meet with investigators to discuss visit findings, any problems and/or issues and actions to be taken, if any. The PI will be responsible for review, follow-up and resolution of monitoring findings.

10 STATISTICAL CONSIDERATIONS

10.1 Sample Size Considerations

The sample size of the study was guided by assumptions regarding the efficacy endpoint. The true underlying shigellosis clinical illness attack rate, as per the BMGF CHIM working group endpoint definition, in the placebo group is estimated to be 60% and the vaccine efficacy is anticipated to be ≥50%. Sample size calculations were performed for 60% attack rates in the placebo group.

In order to be able to detect a vaccine efficacy of 50% with the lower bound of the 90% Confidence Interval at greater than zero with 80% power and an attack rate of 60% among placebo control, 33 subjects per group (vaccinees and controls) are required. Thus, 66 subjects will be enrolled in the CHIM to evaluate the efficacy of SF2a-TT15. Based on the local site facility and staff resources, the 66 subjects will be split into 3 cohorts of 22 subjects. To account for potential drop outs, within each cohort, 30 volunteers will be enrolled in the vaccination phase, thus a total of 90 subjects will be vaccinated in these three cohorts. The excess of 8 subjects per CHIM cohort is based on experience of CVD with previous human challenge studies. (Calculation performed by Chris Gast, Director of Statistics at PATH CVIA, communicated by Calman MacLennan, Senior Program Officer Bacterial Vaccines, Bill and Melinda Gates Foundation)

10.2 Statistical Analysis Plan

Endpoints for Primary Objective. To assess the efficacy of SF2a-TT15 vaccination against Moderate-Severe Shigellosis Illness (*Appendix D*), as elicited by challenge with wild-type *S. flexneri* 2a strain 2457T.

- The point estimate of protective efficacy is to be calculated as the difference in attack rates among placebo recipients and vaccinees divided by the attack rate among placebo recipients. The vaccine efficacy and the 90% and 95% confidence intervals will be calculated.

Endpoints for Secondary Objective 1. To measure the safety and clinical tolerability of two sequential doses of SF2a-TT15.

- The number, proportion, and severity of solicited local and systemic AEs, occurring within the 7 days of vaccination for each of the two doses of vaccine, among vaccine and placebo recipients
- The number, proportion, severity, and relatedness of unsolicited AEs, occurring within 28 days of vaccination for each of the two doses of vaccine, among vaccine and placebo recipients
- The number, proportion, and relatedness of SAEs, occurring at any time during the study, among vaccine and placebo recipients

Endpoints for Secondary Objective 2. To evaluate performance of efficacy of SF2a-TT15 vaccination against different case definitions and endpoint definitions (*Appendix D*), as elicited by challenge with wild-type *S. flexneri* 2a strain 2457T.

- Vaccine efficacy for the prevention of any diarrhea, dysentery, fever, or any combination

of the three criteria (diarrhea, dysentery, and/or fever)
- Vaccine efficacy for the prevention of Severe Shigellosis (according to the CVD Historical Definition)
- The mean and median duration of diarrhea, dysentery, and fever, among vaccine and placebo recipients
- The median and mean number of loose stools (grade 3 or higher), duration of loose stools (grade 3 or higher), cumulative volume of loose stools (grade 3 or higher), maximum 24-hour loose stool output, among vaccine and placebo recipients
- The median and mean time to onset and duration of diarrhea, dysentery, or fever
- The number and proportion of fevers, participants requiring early initiation of antibiotics or intravenous fluids, and participants with vomiting.
- A *Shigella* Disease Severity Score will be calculated for each challenge subject.

Endpoints for Secondary Objective 3. To evaluate the efficacy of SF2a-TT15 vaccination against any positive (qualitative) or quantitative fecal shedding of wild-type *S. flexneri* 2a.
- The geometric mean number (and interquartile range) of the daily and peak quantitative counts (cfu/gram stool), among vaccine and placebo recipients
- The mean and median numbers of days of shedding of the challenge strain
- The area-under-the-curve (AUC), as calculated using the trapezoidal rule, for each challenged participant

Endpoints for Secondary Objective 4. To measure the serum IgG immune responses to SF2a LPS following vaccination and challenge.
- The number and proportion of responders (4-fold increases over baseline) in serum anti-*S. flexneri* 2a LPS IgG ELISA antibody
- The geometric mean titer (GMT), mean fold-rises (compared to baseline), and peak-post-vaccination and post-challenge serum anti-*S. flexneri* 2a LPS IgG ELISA antibody

Endpoints for Secondary Objective 5. To measure the bactericidal activity of SF2a-specific IgG following vaccination and challenge.
- The number and proportion of responders (4-fold increases over baseline) in serum bactericidal activity (SBA) antibody
- The geometric mean titer (GMT), mean fold-rises (compared to baseline), and peak-post-vaccination and post-challenge SBA antibody

Endpoints for Secondary Objective 6. To measure the IgA and IgG ASC and ALS immune responses to SF2a LPS following vaccination and challenge.
- The number and proportion of responders (≥8 SFC) of anti-*S. flexneri* 2a LPS IgG and IgA ASC
- The GMT, mean fold-rises (compared to baseline), and peak post-vaccination and post-challenge anti-*S. flexneri* 2a LPS IgG and IgA ASC
- The number and proportion of responders (≥4-fold increases over baseline) of anti-*S. flexneri* 2a LPS IgG and IgA ALS
- The GMT, mean fold-rises (compared to baseline), and peak post-vaccination and post-challenge anti-*S. flexneri* 2a LPS IgG and IgA ALS

For the endpoints for primary and secondary objectives, the distributions of all measures will be examined and described in terms of sample sizes, means, standard deviations, medians, interquartile ranges, minima and maxima for continuous variables (such as duration of diarrhea,

dysentery, and fever, *Shigella* Disease Severity Score, and geometric mean titers); and counts and proportions for categorical variables (such as the defined endpoints for efficacy [Y/N]), for each group separately. Continuous variables of interest that are not normally distributed will be transformed if needed. To compare the vaccine group with the placebo group, t test or Mann-Whitney U test will be used for continuous variables as appropriate; and Chi-squared test or Fisher's exact will be used for categorical variables as appropriate. All analyses will be performed using Stata/SE version 15.

Exploratory Objectives:

1. To measure pro-inflammatory cytokine markers in stool following challenge.
2. To measure IgG subclasses (IgG1 and IgG2) to SF2a LPS following vaccination and challenge.
3. To measure serum IgA immune responses to SF2a LPS following vaccination and challenge.
4. To measure urinary secretory IgA to SF2a LPS following vaccination and challenge and to store urine samples for potential later analysis of anti-SF2a LPS IgG
5. To measure the IgA and IgG ASC expressing mucosal homing markers (e.g., integrin α4β7-positive) to SF2a LPS following vaccination and challenge
6. To explore the correlates of immunity with protection
7. To compare the immunologic assays performed by different research laboratories
8. To collect, separate and store (at -70° C or colder) PBMC so that in future studies the immune responses to SF2a-TT15 can be further characterized in great detail, including the measurement of T memory and effector cells, B memory cells, homing markers and cytokine production
9. To collect and store serum, urine, and stool specimens for future studies, including but not limited to antibody microarray analysis, fecal 16S rRNA microbiome, transcriptomics, and proteomics.

The general approach to the analysis of serum immunogenicity data (such as IgA and IgG subclasses) will be to calculate the number and proportion of responders (4-fold increases over baseline), the GMT, mean fold-rises (compared to baseline), and peak values post-vaccination and post-challenge. The comparison of the different research laboratories (e.g., TAU and WRAIR ELISA assay data) will be to calculate a rank correlation or compare the percentile rank using sign test. Comparisons in the measures of immunogenicity will be made to the clinical outcomes (endpoints) data to generate potential correlations of protection.

10.3 General Statistical Principles

All participants in the enrolled population who are randomized and receive a masked vaccination will be included in the Full Analysis (FA) Safety Population (n=90). All safety analysis will be performed using this FA safety population. Treatment groups for the safety analysis will be assigned according to the actual treatment received at enrollment. All participants that complete

the inpatient challenge will be included in the Full Analysis (FA) Efficacy Population (n=66). All efficacy and shedding analysis will be performed using this FA efficacy population.

All participants in the FA safety population that received two doses of blinded study product and have no major protocol violations that are determined to potentially interfere with the immunogenicity assessment of the vaccine and who have at least one post-second vaccination immunogenicity assessment will be included in the Per Protocol (PP) Immunogenicity Population. The PP immunogenicity population will serve as the primary analysis population for the immunogenicity endpoints. The criteria for exclusion of participants from the PP population will be established before breaking the blind and will be based on the blinded review of protocol deviations.

In general, all missing data will be treated as missing completely at random and no imputation will be performed except for the safety endpoints, as described below. If some safety data are available for a participant in the FA safety population, but respective secondary endpoint related data are missing, then the participant will be included in the safety analysis and data will be treated as follows for immediate AEs, unsolicited AEs and SAEs.

- If Severity is missing for any AE, then it will be considered as an AE of maximum severity (Grade 3) "Severe", unless it is captured as SAE.
- If "Relationship" is missing, then it will be considered as "Related" to the vaccine administered.
- If, for Start date, the day of event/condition is missing due to any AE, then it will be imputed as the date of last visit.
- If the Stop date of an AE is missing, then it will be treated as ongoing.

For solicited AEs, the following assumptions will be made:

- If dates are missing, but symptoms are reported, then the day post vaccination will be used to calculate the date of the symptom.
- If a symptom is not reported at any time through Day 7, then no imputation for missing data will be performed. The data will be summarized as not reported on the tables.

11 QUALITY CONTROL AND QUALITY ASSURANCE

All clinical trials conducted by the University of Maryland Center for Vaccine Development (CVD) are internally audited for quality assurance (QA) quality control (QC) under the CVD-Wide Quality Management Plan (QMP). The purpose of the QMP is to describe QA/QC procedures designed to ensure site and investigator compliance with applicable regulations, adherence to the IRB-approved protocol, generation of credible, quality data, protect data integrity, and safeguard the safety and well-being of study participants. The plan has the authority to enforce and correct clinical and laboratory deficiencies observed during the conduct of a trial/research study.

Quality Management (QM) activities will proceed in compliance with E6(R2) Good Clinical Practice: Integrated Addendum to ICH E6(R1), March, 2018. Consistent with the University of Maryland Center for Vaccine Development site-wide QMP, a Protocol-specific QMP (Appendix B of the site-wide QMP) will be developed for this protocol. The Protocol-specific plan applies a risk-based approach to QM focused on the essential elements of clinical trial conduct, i.e., recruitment strategies, the informed consent process, eligibility, study-related procedures, clinical and safety assessments, AEs/SAEs, study product management, specimen management, and data management. The plan will also outline site delegation of QC/QA responsibilities, frequency of regulatory file reviews, the audit schedule (e.g., sample size, audit frequency, critical data that will be reviewed, communication, etc.), documentation and reporting of QA findings/queries, and the development of corrective action preventive action (CAPA) plans (as indicated).

12 ETHICS/PROTECTION OF HUMAN SUBJECTS

12.1 Ethical Standard

The investigator will ensure that this study is conducted in full conformity with the principles set forth in The Belmont Report: Ethical Principles and Guidelines for the Protection of Human Subjects of Research of the US National Commission for the Protection of Human Subjects of Biomedical and Behavioral Research (April 18, 1979) and codified in 45 CFR Part 46 and/or the ICH E6; 62 Federal Regulations 25691 (1997).

12.2 Institutional Review Board

The local institution must provide for the review and approval of this protocol and the associated informed consent documents by an appropriate ethics review committee or IRB. Any amendments to the protocol or consent materials must also be approved before they are placed into use unless it is in the best interest of the subjects' safety to implement changes prior to approval. In both the United States and in other countries, only institutions holding a current U. S. Federal-Wide Assurance issued by the Office for Human Research Protections (OHRP) may participate.

Refer to: http://www.hhs.gov/ohrp/assurances/

Prior to enrollment of subjects into this clinical study, the protocol and the informed consent form(s) will be reviewed and approved by the appropriate IRB. Any amendments to the protocol or consent materials will also be reviewed and approved by the appropriate IRB. The responsible official for the IRB will sign the IRB letter of approval of the protocol prior to the start of this clinical study. Should amendments to the protocol be required, the amendments will be submitted to the IRB; an IRB letter of approval of the amendment must be obtained prior to acting upon the amendment in the protocol.

The contact information for the local IRB and the Human Research Protections Office (HRPO) is:

University of Maryland, Baltimore
Human Research Protections Office
620 W. Lexington Street, Second Floor
Baltimore, MD 21201, U.S.A.
Phone 1-410-706-5037

12.3 Informed Consent Process

Informed consent is a process that is initiated prior to the individual's agreement to participate in the study and continues throughout the individual's study participation. Extensive discussion of risks and possible benefits of this therapy will be provided to the participant. Consent forms describing in detail the study, procedures, and risks associated are given to the potential participant and written documentation of informed consent is required prior to starting any study procedure. Consent forms will be IRB-approved and the participant will be asked to read and review the document. Upon reviewing the document, the investigator will explain the research

study to the participant and answer any questions that may arise. The participants will sign the informed consent document prior to any procedures being done specifically for the study. The participant should have the opportunity to discuss the study with their surrogates or think about it prior to agreeing to participate. The participant may withdraw consent at any time throughout the course of the trial. A copy of the informed consent document will be given to the participant for their records. The rights and welfare of the participant will be protected by emphasizing to them that the quality of their medical care will not be adversely affected if they decline to participate in this study.

12.4 Exclusion of Women, Minorities, and Children (Special Populations)

This clinical study will include women, children of 18 years of age and older, and all minorities who meet the inclusion/exclusion criteria, regardless of religion, sex, or ethnic background.

12.5 Subject Compensation

Thoughtful consideration has been made toward subject compensation for healthy volunteer participants enrolled into clinical studies involving non-licensed vaccines and experimental infections. The overarching ethical issues which the CVD's compensation scheme attempts to address are: (1) avoidance of "undue inducement", (2) payments which may result in economically disadvantaged populations bearing an overwhelming share of the risks and burdens of research, and (3) payments which may violate the ethical norms of the investigator-subject relationship by turning it into a commercial relationship. The compensation scheme for this study is adopted from the Wage-Payment Model.[52] Under the Wage-Payment Model, a participant is assumed to require little skill, but does require time, effort, and an expectation of experiencing undesirable or uncomfortable procedures. Therefore, the CVD's compensation rates are calculated to approximate the hourly wages of an unskilled high-risk laborer. The compensation rate used in this study is an standard rate that is used across all of CVD's clinical research. This compensation rate is clearly described in the informed consent form and is unlikely to be unduly coercive.

12.6 Subject Confidentiality

Subject (participant) confidentiality is strictly held in trust by the investigators, their staff, and the Sponsor. This confidentiality is extended to cover testing of biological samples and other testing in addition to the clinical information relating to participant.

The study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study, or the data, will be released to any unauthorized third party without prior written approval of the sponsor.

The study monitor, or other authorized representatives of the sponsor, may inspect all documents and records required to be maintained by the investigator including, but not limited to, medical

records (office, clinic, or hospital) and pharmacy records for the participants in this study. The clinical study site will permit access to such records.

Participant identity data will be contained in paper study records which will be kept in a locked file cabinet and in a secure electronic database, accessible only to authorized users at each clinical site. The study database will be user-restricted and password-protected. The study database will identify participants by a coded study Volunteer ID number assigned by clinical site personnel, thus participants will not be identified by name.

Subject confidentiality and the limits for access to records is described in the informed consent form.

12.7 Future Use of Stored Specimens

It is intended that any remaining specimens at the closure of the study will be stored at the CVD or partners (i.e., WRAIR, Institut Pasteur, or Tel Aviv University) and may be used for further immunological analyses. Samples that are sent to Institut Pasteur may be stored for up to 15 years. All other samples may be stored indefinitely. Participants may choose to have their specimens destroyed and must do this in a written request. This is described in the consent form.

13 ACCESS TO SOURCE DATA/DOCUMENTS

Source data includes all information, original records of clinical findings, observations, or other activities in a clinical trial necessary for validation of the clinical data. Examples of these original documents and data records include, but are not limited to, hospital records, clinical and office charts, laboratory notes, memoranda, subjects' memory aid or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate and complete, microfiches, photographic negatives, microfilm or magnetic media, x-rays, and subject files and records kept at the pharmacy, at the laboratories, and medico-technical departments involved in the clinical trial. All information on the DCFs will be traceable to these source documents, which are maintained in the subject's study file.

The PI is responsible for ensuring the accuracy, completeness, legibility, and timeliness of the data reported. Source documents are maintained for recording data for each subject enrolled in this clinical study. Study subjects' data collected on the DCF during the trial will only be identified by subject number. If, as an exception, it is necessary for safety or regulatory reasons to identify the patient, both the Sponsor and the Investigator are bound to keep this information confidential.

All the information required by the protocol should be provided; any omissions require explanation. Source documents and DCFs should be completed and available for monitoring and/or collection within a timely manner so that the monitor may check the entries for completeness, accuracy and legibility, and ensure that the DCFs are signed by the Investigator, and ready for transmission to the Sponsor.

All source documents and DCFs should be completed in a neat, legible manner to ensure accurate interpretation of data. Black or blue ink is required to ensure clarity of reproduced copies. When making a change or correction, cross out the original entry with a single line and initial and date the change. DO NOT ERASE, OVERWRITE OR USE CORRECTION FLUID OR TAPE ON THE ORIGINAL.

The Investigator or designee must enter the information required by the protocol onto the DCF provided. The Sponsor's clinical site monitor will review the DCF for completeness and accuracy and instruct site personnel to make any required corrections or additions.

14 DATA HANDLING AND RECORD KEEPING

The site PI is responsible for ensuring the accuracy, completeness, legibility, and timeliness of the data reported.

Source data will be generated by the site and collected on data collection forms. Source data will be entered into a REDCap data management system, managed by the Clinical and Translational Research Informatics Center (CTRIC), University of Maryland School of Medicine. REDCap is a secure web-based application for building an electronic database that provides a 21 CFR Part 11, FISMA, and HIPAA-compliant environment. Quality control audits of all key safety, laboratory, and clinical data in the database will be made after data entry has been completed. Coexistent medical conditions, AEs and other medical events will be coded using MedDRA dictionary. Concomitant medications will be coded using WHO-DD dictionary. When the database has been declared to be complete and accurate, the database will be locked. Any changes to the database after that time will only be by joint written agreement of the study team.

Data reported in REDCap are derived from the data collection forms and should be consistent with the data collection forms or the discrepancies should be explained.

The sponsor and/or its designee will provide guidance to the site PIs and other study personnel on making corrections to the data collection forms.

As part of the development program for the *Shigella* vaccine candidate, unblinded coded data (including treatment assignment; unblinded safety, immunogenicity, and challenge results data; and unblinded reports for the DSMB) collected from this clinical trial will be shared between unblinded teams of the sponsor and the vaccine developer.

After the adjudication of the challenge phase results, the vaccine developer (Institut Pasteur) will be unblinded. Up to three members of Institut Pasteur will have access to the unblinded data for the purpose of in-depth analysis of the immunogenicity data and challenge outcomes overall, per cohort, and per volunteer. Considering the global need for a *Shigella* vaccine, rapid assessment of the potential efficacy of the vaccine candidate is required to move the vaccine approach forward.

At Institut Pasteur, all data will be stored on a secure server accessible with a login and password to which only the unblinded persons have access. The risk of impact on the study is minimized as the Institut Pasteur personnel is not involved in the clinical evaluation and management of the participants. Furthermore, unblinding of Institut Pasteur staff will occur during the follow up phase of the trial, the treatments and challenges have already been completed. Guidelines for information sharing with blinded project team members, particularly at the sponsor and the laboratories performing the safety and immunogenicity analyses, are in place at Institut Pasteur.

The data will be shared with the Bill & Melinda Gates Foundation and the Gates Medical Research Institute as they are partners of the Institut Pasteur *Shigella* vaccine candidate development. These data may comprise aggregated data but also per subject analyses. Subject codes will not be included in shared data, so that the blinding of the treatment of the individual subjects will be maintained. Aggregated data might also be shared with the sponsor.

15 IN SUMMARY, EXCEPT THE PHARMACIST AND THE UNBLINDED STATISTICIAN AT THE SPONSOR, NONE OF THE INDIVIDUALS INVOLVED IN THE STUDY (PARTICIPANTS, CLINICAL AND LABORATORY STAFF, SPONSOR PERSONNEL) WILL HAVE ACCESS TO TREATMENT ASSIGNMENT. DATA WILL BE UNBLINDED AFTER ALL SUBJECTS WILL HAVE COMPLETED THE STUDY AND THE DATABASE LOCK WAS COMPLETED OR IN CASE OF EMERGENCY. PERSONNEL AT THE VACCINE DEVELOPER WHO ARE NOT INVOLVED IN STUDY CONDUCT WILL BE UNBLINDED AFTER THE ADJUDICATION FOR DECISION MAKING FOR PROGRESSION OF THE *SHIGELLA* VACCINE DEVELOPMENT. PUBLICATION POLICY

All investigators funded by the Bill & Melinda Gates Foundation must agree to their Open Access Policy. https://www.gatesfoundation.org/How-We-Work/General-Information/Open-Access-Policy

The Foundation's Open Access Policy contains the following elements:

1. Publications Are Discoverable and Accessible Online. Publications will be deposited in a specified repository(s) with proper tagging of metadata.
2. Publication Will Be On "Open Access" Terms. All publications shall be published under the Creative Commons Attribution 4.0 Generic License (CC BY 4.0) or an equivalent license. This will permit all users of the publication to copy and redistribute the material in any medium or format and transform and build upon the material, including for any purpose (including commercial) without further permission or fees being required.
3. Foundation Will Pay Necessary Fees. The Foundation will pay reasonable fees required by a publisher to effect publication on these terms.
4. Publications Will Be Accessible and Open Immediately. All publications shall be available immediately upon their publication, without any embargo period.
5. Data Underlying Published Research Results Will Be Accessible and Open Immediately. The Foundation will require that data underlying the published research results be immediately accessible and open.

Therefore, following completion of this clinical trial, the investigators intend to publish the results of this research in a peer-reviewed scientific journal, within 12 months of locking the database. The International Committee of Medical Journal Editors (ICMJE) member journals have adopted a trials-registration policy as a condition for publication. This policy requires that all clinical trials

be registered in a public trials registry, such as ClinicalTrials.gov* (http://clinicaltrials.gov/), which is sponsored by the National Library of Medicine.

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APPENDIX A1: SCHEDULE OF EVENTS, CHALLENGE SUBJECTS (COHORTS 1-3)

CHALLENGE (n=66)						admit											discharge				
Study Day	Sc	1	8	29	36[a]	55[a]	56[a]	57[a]	58[a]	59[a]	60[a]	61[a]	62[a]	63[a]	64[a]	65[a]	66[a]	69[a]	85[a]	141[a]	237[b]
Consent, Review Eligibility	x																				
Screening/Safety Clinical Labs	25 mL				10 mL																
Screening Stool Culture‡					x																
urine pregnancy, prior vaccine/challenge		x		x				x													
Vaccination		**#1**		**#2**																	
Challenge (3-days **A**ntibiotics)								**C**					A	A	A						
Memory Aid, distribute & collect		d	c	d	c																
Adverse Events*		x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Stool Cultures							x#	x	x	x	x	x	x	x	x	x	x	x**	x**		x**
1) Serum: ELISA antibody, SBA, subclasses		15	15	15	15		15#								15				15	15	15
2a) Fresh PBMC: ASC & ALS (all 22 vols/cohort)		20	20	20	20		20#								20						
2b) Fresh PBMC: ASC & ALS Homing Subset (up to 8/cohort)†			30		30										30						
3a) cryopreserve PBMC: Teff/Tm cells, memory B (all 22)		90		90			90#												90	90	90
3b) cryopreserve PBMC: pTfh (not in Homing)†											30				30						
4) Stool: IgA ELISA		x		x			x#												x	x	x
5) urine: for sIgA/IgG (Dani Cohen analysis)		x		x			x#								x				x	x	x
6) whole blood (PaxGene) for transcriptomics								2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5				2.5		
serum banking for microarray		x	x	x	x		x#								x				x	x	x
stool (no preservative) for cytokine analysis								x	x	x	x	x	x	x	x						
stool banking (RNALater) for microbiome, transcriptomics, etc.		x		x			x#	x	x	x	x	x	x	x	x	x	x		x	x	x

‡ *instead of stool culture, it is acceptable to use BioFire GI Panel (or equivalent molecular diagnostic test)*

* *Unsolicited AEs will be reported from enrollment through 28 days after each dose of vaccine, SAEs will be reported from enrollment through study completion (i.e., Day 1 through Day 237)*

** *Only qualitative stool cultures will be performed on Days 69, 85 and 237 (only on participants that were challenged)*

† *Up to eight volunteers per cohort (i.e., up to twenty-four total) will be randomly selected to participate in the "Homing Studies Subset" and will be requested to provide an additional volume of blood for homing studies (i.e., Days 8, 36, and 64); these volunteers will also be preferentially selected to participate in the challenge phase of the study. On challenge days, only a maximum of six volunteers will contribute to the homing study (Day 64). Challenge participants not in the Homing Studies Subset will provide blood 3 days and 7 days post-challenge (i.e., Days 60 and 64) for peripheral T follicular helper cell (pTfh) studies.*

\# *These indicated research blood, stool, and urine specimens may be collected at any point prior to challenge, during acclimatization (i.e., Days 55, 56, or 57)*

Note: Some specified visit days (and windows) are intended to be relative to timing of 2nd vaccination ([a]) or challenge ([b])

APPENDIX A2: SCHEDULE OF EVENTS, VACCINE-ONLY SUBJECTS (COHORTS 1-3)

VACCINATION only (n=24)

Study Day	Sc	1	8	29	36[a]	57[a]	85[a]	141[a]	237[a]
Consent, Review Eligibility	x								
Screening/Safety Clinical Labs	x				10 mL				
Screening Stool Culture‡					x				
urine pregnancy, prior vaccine/challenge		X		x					
Vaccination		**#1**		**#2**					
Memory Aid, distribute & collect		d	c	d	c				
Adverse Events*		X	x	x	x	x	x	x	x
1) Serum: ELISA antibody, SBA, subclasses		15	15	15	15	15	15	15	15
2a) Fresh PBMC: ASC & ALS (all 22 vols/cohort)		20	20	20	20				
2b) Fresh PBMC: ASC & ALS Homing Subset (up to 8/cohort)†			30		30				
3a) cryopreserve PBMC: Teff/Tm cells, memory B		90		90		90	90	90	90
4) Stool: IgA ELISA		X		x		x	x	x	x
5) urine: for sIgA/IgG (Dani Cohen analysis)		X		x		x	x	x	x
serum banking for microarray		X	x	x	x	x	x	x	x
stool banking (RNALater) for microbiome, etc.		X		x		x			

‡ *instead of stool culture, it is acceptable to use BioFire GI Panel (or equivalent molecular diagnostic test)*

* *Unsolicited AEs will be reported from enrollment through 28 days after each dose of vaccine; SAEs will be reported from enrollment through study completion (i.e., Day 1 through Day 237)*

† *It is intended that the up to eight volunteers per cohort (i.e., up to twenty-four total) which are randomly selected to participate in the "Homing Studies Subset" will be preferentially selected to participate in the challenge phase of the study. Nonetheless the volunteer cannot be compelled to participate in the challenge phase and therefore there is no Day 64 blood draw for homing studies if they do not participate in the challenge phase.*

Note: Some specified visit days (and windows) are intended to be relative to timing of 2nd vaccination ([a])

APPENDIX B: SCREENING TESTS

Analyte	Unit	Acceptable Values for Screening Labs:
White Blood Cell count (WBC)	thou/mcL	3600–11000 (2800–11,000 for African-Americans)
Absolute Neutrophil Count (ANC)	thou/mcL	1500–8000 (1200-8000 for African-Americans)
Hemoglobin (Females)	g/dL	F: 11.5–16.5
Hemoglobin (Males)	g/dL	M: 13.0–18.0
Platelet count	per mm^3	125,000–450,000
ALT	IU/L	<50
Creatinine (females)	mg/dL	< 1.6
Creatinine (males)	mg/dL	< 1.6
Bilirubin, total	mg/dL	< 1.4 (unless known Gilbert's syndrome, then <2.0)
Glucose - urinalysis	n/a	trace or negative
Protein - urinalysis	n/a	≤ 1+
Occult blood - urinalysis	n/a	trace or negative*
Urine pregnancy test	n/a	Negative
Serum β-HCG	n/a	Negative
Hepatitis B surface antigen	n/a	Negative
Hepatitis C virus ELISA	n/a	Negative**
HIV	n/a	Negative
Stool Culture	n/a	No *Salmonella*, *Shigella*, *Campylobacter*, *Yersinia* or *Vibrio* (The absence of normal flora or "no normal enteric flora present" is not acceptable)
BioFire GI Panel (or equivalent molecular diagnostic test)	n/a	No *Salmonella*, *Shigella/EIEC*, *Campylobacter*, *Yersinia, Vibrio, or Norovirus GI/GII.*
n/a, not applicable *presence is acceptable for menstruating women ** instances where there is an "indeterminate" HCV antibody screening result may be acceptable if the participant can document a negative Hepatitis C virus viral load (e.g., undetected viral load or no measurable virus in a HCV RNA quantitative test)		
12-lead Electrocardiogram	Must <u>not</u> have any of the following, in order to be acceptable: • Pathologic Q wave abnormalities • Significant prolonged QT • Significant ST-T wave changes • Left ventricular hypertrophy • Right bundle branch block • Left bundle branch block • Advanced A-V heart block • Non-sinus rhythm, excluding isolated premature atrial contractions	

APPENDIX C: CLINICAL SAFETY LAB TOXICITY

Test	Mild Grade 1	Moderate Grade 2	Severe Grade 3	Potentially Life Threatening Grade 4
WBC, increase	1.1 – 1.5 xULN	1.6 – 2.0 xULN	2.1 – 2.5 xULN	≥ 2.6 xULN
WBC, decrease*	1.1 – 1.5 xLLN	1.6 – 2.0 xLLN	2.1 – 2.5 xLLN	≥ 2.6 xLLN
ANC decrease	1.1 – 1.5 xLLN	1.6 – 2.0 xLLN	2.1 – 2.5 xLLN	≥ 2.6 xLLN
Hemoglobin decrease	1.1 – 1.5 xLLN	1.6 – 2.0 xLLN	2.1 – 3.0 xLLN	>3 xLLN
Platelet count	1.3 – 1.5 xLLN	1.6 – 2.0 xLLN	2.1 – 3.0 xLLN	>3 xLLN
Sodium, low	132 -134	130 - 131	125 - 129	< 125
Sodium, high	146 – 148	149 - 150	151 - 152	> 152
Potassium, high	5.6 – 5.8	5.9 – 6.1	6.2 – 6.5	> 6.5
Potassium, low	3.3 – 3.4	3.1 – 3.2	2.9 – 3.0	< 2.9
Creatinine*	1.1 – 1.5 xULN	1.6 – 2.0 xULN	2.1 – 3.0 xULN	> 3.0 xULN
AST (SGOT)	1.6 - 2.5 xULN	2.6 – 4.0 xULN	4.1 – 10.0 xULN	>10 xULN
ALT (SGPT)	1.6 - 2.5 xULN	2.6 – 4.0 xULN	4.1 – 10.0 xULN	>10 xULN
Alkaline Phosphatase	1.6 - 2.0 xULN	2.1 – 3.0 xULN	3.1 – 10.0 xULN	>10 xULN
Total Bilirubin	1.2 - 1.5 xULN	1.6 – 2.0 xULN	2.1 - 4.0 xULN	>4 xULN

ULN = upper limit of normal range

LLN = lower limit of normal range

*if the baseline is outside of the reference range (e.g., African-American acceptable value), then relative changes to the baseline value will be evaluated for toxicity

APPENDIX D: ENDPOINT DEFINITIONS FOR SHIGELLOSIS

Definition of Loose Stool by the Grading of Stool Consistency				
normal stool		*loose or diarrheal stool*		
Grade 1	**Grade 2**	**Grade 3**	**Grade 4**	**Grade 5**
Well formed; does not take the shape of the container	Soft; does not easily take the shape of the container	Thick liquid stool; easily takes the shape of the container	Opaque watery diarrheal stool	Clear watery or "rice water" diarrheal stool

Primary Endpoints, Definitions for Moderate-Severe Shigellosis Illness (according to the BMGF CHIM Working Group's case definition for moderate-severe shigellosis, any one of the below will satisfy as a primary endpoint)	
Severe Diarrhea	≥6 loose stools in 24 hours **OR** >800 grams of loose stools in 24 hours
Moderate Diarrhea	[4-5 loose stools in 24 hours **OR** 400-800 grams of loose stools in 24 hours] **AND** [oral temperature ≥38.0˚C (100.4°F)† **OR** ≥1 moderate constitutional/enteric symptom‡ **OR** ≥2 episodes of vomiting in 24 hours]
Dysentery	≥2 loose stools with gross blood (confirmed by fecal occult blood test) in 24 hours **AND** [oral temperature ≥38.0˚C (100.4°F)† **OR** ≥1 moderate constitutional/enteric symptom‡ **OR** ≥2 episodes of vomiting in 24 hours]

† Oral temp must be confirmed by two separate readings at least five minutes apart

‡ Constitutional/enteric symptoms include: nausea, abdominal pain, abdominal cramping, myalgia, arthralgia, and malaise; "moderate" severity is defined as causing interference with routine activities; "severe" severity symptom is defined as causing the inability to perform routine daily activities. Anorexia, rigors/chills, tenesmus/fecal urgency, gas/flatulence, and headache are not included symptoms for the endpoint.

Secondary Endpoints, Definitions for Shigellosis Illness (according to previously used CVD case definitions for shigellosis)	
Diarrhea	≥2 loose stools totaling ≥200 grams in 24 hours **OR** a single loose stool of >300 grams
Dysentery	≥1 loose stool containing gross blood (confirmed by fecal occult blood test)
Fever	2 oral temperatures ≥37.8°C (100˚F), separated by at least 5 minutes apart
Any illness	Satisfies any one of the three above criteria
CVD historical definition of Severe Shigellosis	The criteria for both diarrhea and dysentery, according to the CVD definitions (above), are met **AND** oral temperature of ≥38.9°C (102°F)† **AND** ≥10 loose stools have been passed

† Oral temp must be confirmed by two separate readings at least five minutes apart

APPENDIX E: CRITERIA FOR THE EARLY INITIATION OF ANTIBIOTICS

Events which trigger early antibiotic treatment (any one of the below criteria)
The criteria for a primary endpoint of moderate-severe shigellosis (BMGF CHIM definitions) are met
The criteria for the CVD historical definition of Severe Shigellosis are met
Oral temperature of ≥39°C (102.2°F)[†]
Principal Investigator (PI or clinical designee) discretion, considering the overall safety and welfare of the participant

[†] Oral temp must be confirmed by two separate readings at least five minutes apart

APPENDIX F: SAMPLE FORM FOR THE COLLECTION OF SUBJECTIVE SYMPTOMS WHICH CONTRIBUTE TO THE SHIGELLA DISEASE SEVERITY SCORE

Shigella CVD 30000 Inpatient - Data Collection Form Volunteer ID: _____

Post-Challenge, Inpatient Daily Assessment

(CIRCLE ONE) Day 1(Challenge) Day 2 Day 3 Day 4 Day 5
Day 6 Day 7 Day 8 Day 9 Day 10

The information on this form reflects the events from the 24 hour period of the date indicated. The form is completed on the following day by the investigator.

Date of Events: __ __ / __ __ __ / __ __ __ __ (dd/MMM/yyyy)

DAILY REACTOGENICITY/SOLICITED ADVERSE EVENTS				
Objective Symptoms	**0**	**1**	**2**	**3**
Diarrhea[a]	☐	☐	☐	☐
Dysentery[b]	☐	☐	☐	☐
Vomiting[c]	☐	☐	☐	☐
Fever[d]	☐	☐	☐	☐
Subjective Symptoms				
Abdominal Pain/Cramping	☐	☐	☐	☐
Tenesmus	☐	☐	☐	☐
Malaise/Fatigue	☐	☐	☐	☐
Nausea	☐	☐	☐	☐
Anorexia/Loss of Appetite	☐	☐	☐	☐
Myalgia/Body Ache	☐	☐	☐	☐
Arthralgia/Joint Ache	☐	☐	☐	☐
Headache	☐	☐	☐	☐

[a] **Diarrhea Scale:**
0 = None (or not achieving mild definition)
1 = Mild (≥2 loose stools of ≥ 200 mL in 24 hr *OR* single loose stool of ≥ 300 mL)
2 = Moderate (4-5 loose stools in 24h *OR* 400-800 mL loose stools in 24h)
3 = Severe (>6 loose stools in 24h *OR* > 800 mL loose stools in 24h)

[b] **Dysentery Scale:**
0 = None
1 = 1
2 = 2-3
3 = 4 or more

[c] **Vomiting Scale:**
0 = None
1 = 1-2 episodes/24h
2 = 3-4 episodes/24h
3 = 5 or more/24h

[d] **Fever Scale:**
0 = None
1 = 100.0-100.3°F or 37.8-37.9 °C
2 = 100.4-101.9°F or 38.0-38.8°C
3 = ≥102.0°F or ≥38.9°C

General Grading Scale:
0 = None
1 = Mild, no interference
2 = Moderate, some interference
3 = Severe, significantly interferes

Did any unsolicited Adverse Events occur (anything not indicated above)	☐ No	☐ Yes
Were there any changes in concomitant medications?	☐ No	☐ Yes
Was there any grade 3 or higher stool(s) passed in these 24h?	☐ No	☐ Yes
Were antibiotics initiated on this day?	☐ No	☐ Yes

Comments: _____

Signature: Date (dd/MMM/yyyy):

1. _____ _____
2. _____ _____
3. _____ _____

Version 0.1, 27 Mar 2019