Statistical Analysis Plan

Study 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

Protocol Number: Shigella CVD 30000

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Study Synopsis

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 one hundred and two 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.

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 6 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					
				No. Subjects anticipated with 1:1 randomization	
Cohort	Setting	No. doses	Target Enrollment	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

A. Objectives and Endpoints

1. Primary Objective and Endpoints

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 (of achieving the primary endpoint for moderate-severe shigellosis illness) 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.

2. Secondary Objectives and their Endpoints

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, separately analyzing with all participants, with only challenge participants, and with only vaccine (no challenge) participants for response rates to vaccination (through Day 57) and challenge (post-Day 57 for challenge participants).

- The geometric mean titer (GMT), mean fold-rises (compared to baseline), median titer, and peak-post-vaccination and post-challenge serum anti-*S. flexneri* 2a LPS IgG ELISA antibody, separately analyzing with all participants, with only challenge participants, and with only vaccine (no challenge) participants.

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), median titer, and peak-post-vaccination and post-challenge SBA antibody

Endpoints for Secondary Objective 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.

- The number and proportion of responders (≥8 spot-forming cell [SFC]) of anti-*S. flexneri* 2a LPS IgG and IgA ASC
- The GMT, mean fold-rises (compared to baseline), median titer, 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), median titer, and peak post-vaccination and post-challenge anti-*S. flexneri* 2a LPS IgG and IgA ALS

3. Exploratory Objectives and their Endpoints

Endpoint for Exploratory Objective 1. To measure pro-inflammatory cytokine markers in stool following challenge.

- The GMT, mean fold-rises (compared to baseline), median titer, and peak post-challenge myeloperoxidase (MPO) and calprotectin level.[†]

[†]According to the commercial kit specifications, the human fecal myeloperoxidase (MPO) sandwich ELISA[1] produces a sensitivity/LLOD of 0.15 µg/mL and dynamic range of 0 – 256 µg/mL; the human calprotectin sandwich ELISA[2] produces a sensitivity/LLOD of 2.5 ng/mL and dynamic range of 0 – 2000 µg/mL.

Exploratory Objective 2. To measure IgG subclasses (IgG1 and IgG2) to SF2a LPS following vaccination and challenge. *The plan will be to define this analysis after the preliminary efficacy data have been reviewed.*

Exploratory Objective 3. To measure serum IgA 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 IgA ELISA antibody, separately analyzing with all participants, with only challenge participants, and with only vaccine (no challenge) participants.

[1] Epitope Diagnostics, MPO kit KT-844. http://www.epitopediagnostics.com/kt844
[2] Epitope Diagnostics, Calprotectin kit KT-849. http://www.epitopediagnostics.com/kt849

- The geometric mean titer (GMT), mean fold-rises (compared to baseline), median titer, and peak-post-vaccination and post-challenge serum anti-*S. flexneri* 2a LPS IgA ELISA antibody, separately analyzing with all participants, with only challenge participants, and with only vaccine (no challenge) participants.

Exploratory Objective 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.

- The number and proportion of responders (4-fold increases over baseline) in urine anti-*S. flexneri* 2a LPS sIgA ELISA antibody.
- The geometric mean titer (GMT), mean fold-rises (compared to baseline), median titer, and peak-post-vaccination and post-challenge urine anti-*S. flexneri* 2a LPS sIgA ELISA antibody.
- *Note: The urine specimens from challenge participants are intended to be analyzed. Non-challenge participant urine specimens may be analyzed with additional independent funding (i.e., they have not been budgeted under the current funding).*

Exploratory Objective 5. To measure the IgA and IgG antibody secreting cells (ASC) expressing mucosal homing markers (e.g., integrin α4β7-positive) to SF2a LPS following vaccination and challenge.

- The number and proportion of responders (≥8 spot-forming cell [SFC]) of anti-*S. flexneri* 2a LPS IgG and IgA ASC expressing mucosal homing markers
- The GMT, mean fold-rises (compared to baseline), median titer, and peak post-vaccination and post-challenge anti-*S. flexneri* 2a LPS IgG and IgA ASC expressing mucosal homing markers.

Exploratory Objective 6. To explore the correlates of immunity with protection.

- This analysis will be conducted with the cumulative challenge data and separately per cohort (cohort 1, 2, and 3 to be analyzed separately).

Exploratory Objective 7. To compare the immunologic assays performed by different research laboratories.

Exploratory Objective 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 T memory and effector cells, B memory cells, homing markers and cytokine production.

Exploratory Objective 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.

B. Statistical Methods

For the endpoints for primary and secondary efficacy objectives (not for the immunogenicity objectives), the distributions of all measures will be examined graphically and described in terms of sample sizes, means (or geometric means when applicable), standard deviations, median, interquartile ranges, minima and maxima for continuous variables (such as duration of diarrhea, dysentery, and fever, *Shigella* Disease Severity Score); and counts and proportions for categorical variables (such as the defined endpoints for efficacy [Y/N]), for each group separately. When computing geometric means, zeros or those values that are below the limit of detection will be replaced by one half the limit of detection (e.g., less than 1 becomes 0.5). 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 16.

The general approach to the analysis of 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, standard deviations, interquartile ranges, confidence intervals, mean fold-rises (compared to baseline), median titers, and peak values post-vaccination and post-challenge. We will conduct the analysis for all participants and by challenge group (i.e., those who do not get challenge vs. those who do get 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.

C. Safety Reports

The frequency and timing of safety reports which are to be made available to the Data and Safety Monitoring Board (DSMB) is to be defined by the finalized DSMB Charter. The tentatively planned data review meetings are as follows:

- DSMB review meeting #1. Review of 7-days safety information post-first dose of vaccine for Cohort 1.
- DSMB review meeting #2. Review of post-challenge safety information, after discharge of Cohort 1, and 7-days safety information post-first dose of vaccine for Cohort 2.
- DSMB review meeting #3. Review of 7-days safety information post-first dose of vaccine for Cohort 3.
- DSMB review meeting #4. Review of post-challenge safety information, after discharge of Cohort 2.
- DSMB review meeting #5. Review of post-challenge safety information, after discharge of Cohort 3.

The information to be presented to the DSMB in these Safety Reports are to include the following: subject demographics, enrollment and compliance table, vaccine solicited adverse events table, and adverse events listing. Mock-up example tables and figure provided. The DSMB may request additional information beyond this information provided.

Table 1: Subject Demographics			
	Cohort 1	**Cohort 2**	**Cohort 3**
Total Enrolled			
Gender,			
No. male (%)			
Ethnicity,			
No. non-Hispanic (%)			
Race			
Black/African-American			
White			
Asian			
Am Indian/Alaska Native			
Pacific Islander/Hawaiian			
Multi-race			
Unknown or not reported			

Table 2: Enrollment and Compliance			
	Cohort 1	**Cohort 2**	**Cohort 3**
No. Screened			
No. Enrolled (Dose #1), Day 1			
No. Complete Day 8 Visit			
No. Completing Dose #2, Day 29			
No. Completing Day 36 Visit			
No. Completing Challenge, Day 57			
No. Unchallenged, Day 57 Visit*			
No. Completing Day 85 Visit			
No. Completing Day 141 Visit			
No. Completing Day 237 Visit			

**number of unchallenged subjects completing the Day 57 visit*

Table 3: Vaccine Solicited Adverse Events, maximum severity during the 7 days post-dose #1													
	Cohort 1					Cohort 2					Cohort 3			
grade	0	1	2	3		0	1	2	3		0	1	2	3
Systemic Symptoms														
Fever*														
Headache														
Fatigue/Malaise														
Arthralgia														
Myalgia														
Diarrhea														
Anorexia														
Chills														
Vomiting														
Local Symptoms														
Pain, injection site														
Erythema														
Induration														

**Fever defined as ≥38.0°C is coded as grade 1*

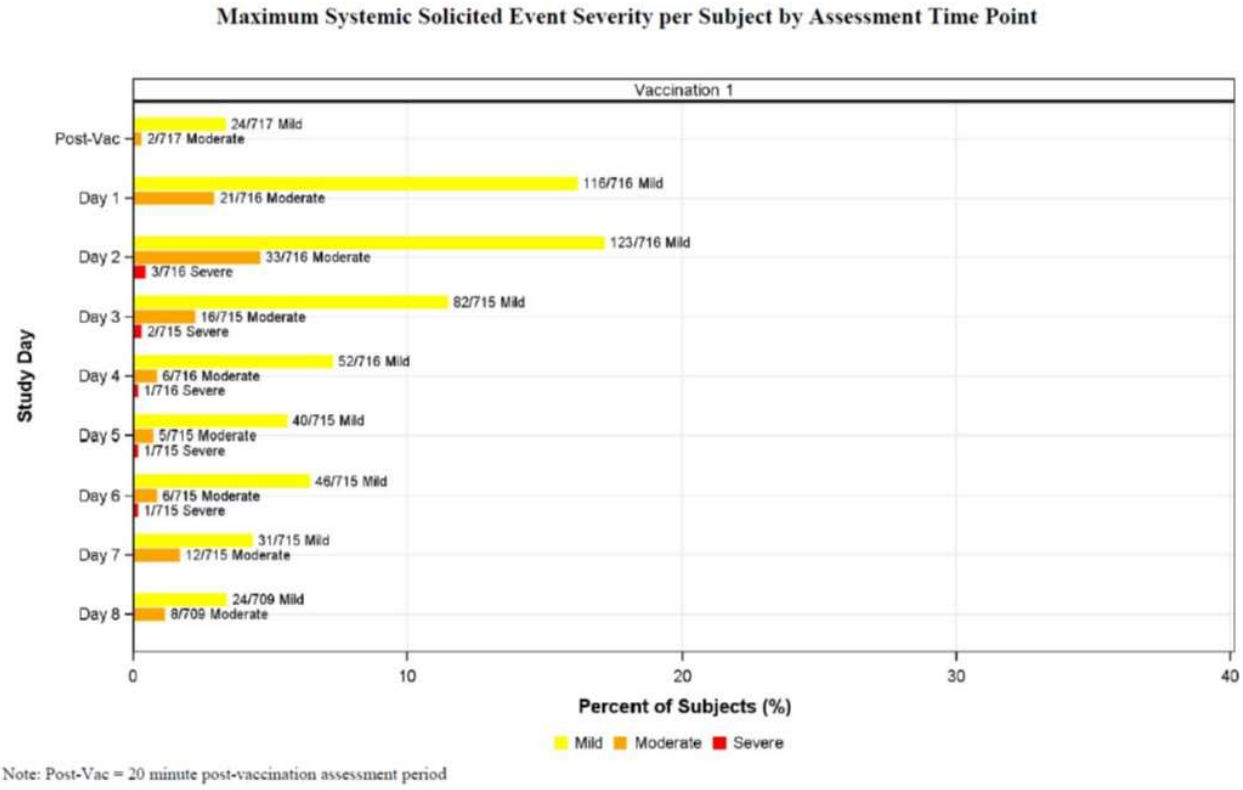
Table 4: Listing of Serious Adverse Events							
No.	Subject ID	No. days post-vaccination	Duration (days)	Reason reported as SAE	Severity	Relation to Vaccination	SAE description or alternate etiology
1							
2							
3							

Table 5: Non-solicited Adverse Event Line Listing						
Subject ID	Cohort	No. days post-vaccination	Outcome*	AE Term	Severity	Relationship

*Recovered/resolved, recovered with sequelae, or ongoing

Figure 1: Maximum Systemic or Local Solicited Adverse Event Severity per Subject by Timepoint

[it is intended that an individual figure represent each systemic and local solicited AE]



Note: Post-Vac = 20 minute post-vaccination assessment period

D. Interim efficacy communications to BMGF

As part of the grant set up, the Bill & Melinda Gates Foundation (BMGF) requested to be informed about the outcome of the challenge after each cohort. This information will also be shared with the Gates Medical Research Institute (Gates MRI).

Challenge outcome:

- Number of subjects reaching the primary endpoint
- Attack rate in the placebo group

S. flexneri 2a dose administered for the challenge:

- Target dose:
- Determined dose:

E. Adjudication Board

After the completion of the study challenges and interim locking of the database through Day 66 of Cohort 3, an Adjudication Board will be assembled to review and to make determinations on the clinical outcomes of the trial. Adjudication Board members are intended to include available members from CVD, WRAIR/NMRC, Institut Pasteur, and TAU (who are not involved in the immunogenicity analyses). The timing of this review may limit available persons for this review, so a minimum of 3 persons must attend in order to provide a tie-breaker decision. Additional participation may be extended (subject to availability) to *Shigella* challenge experts from either Cincinnati Children's Hospital Medical Center and/or Johns Hopkins University.

A set of data slides will be complied and presented to the Adjudication Board which will summarize subject-by-subject information regarding diarrheal stools (weights, number, and times of onset), fevers, and selected subjective symptoms as part of shigella illness. These data will be presented as blinded data. Each member of the Adjudication Board will complete a form to document their independent determination regarding each subject's achievement for satisfying each of the protocol-defined clinical endpoints.

A mocked-up Adjudication Board Form is presented, below.

					Max Severity of Symptom (0, 1, 2, or 3)										
Subject ID	Max Vol Loose Stools in 24 hr (grams)	Max No. Loose Stools in 24 hr	≥2 loose stools with positive occult blood in 24 hr (Y/N)	Max Temp (°F)	Fever	Abd pain/cramp	Anorexia	Arthalgia	Myalgia	Headache	Nausea	Vomiting	Stool Culture Positive for Shigella (Y/N)		Satisfied Primary Endpoint (Y/N)	comments

Board Member
Printed Name: _____ Signature: _____ Date: _____

F. Interim Analysis Report 1: Preliminary Efficacy Readout

After the completion of the challenge of the third cohort, an interim analysis report for a preliminary efficacy readout is to be generated. This interim report will be shared with the BMGF based on their request during the grant preparation and with the Gates MRI and will provide critical information for a go/no go decision by BMGF and the Gates MRI for further development of a multivalent *Shigella* vaccine based on the Institut Pasteur synthetic carbohydrate-based conjugate approach.

The interim analysis report is to consist of cumulative safety information (i.e., the three tables plus line listing of adverse events) and preliminary calculations of the efficacy of the vaccine.

Table 5: Cumulative Subject Demographics			
	All	**Challenged**	**Not Challenged**
Total Enrolled			
Gender,			
No. male (%)			
Ethnicity,			
No. non-Hispanic (%)			
Race			
Black/African-American			
White			
Asian			
Am Indian/Alaska Native			
Pacific Islander/Hawaiian			
Multi-race			
Unknown or not reported			

Table 6: Calculated Vaccine Efficacy				
	Vaccine	**Placebo**	**VE* (95% CI)**	**VE* (90% CI)**
Total No. Challenged				
No. Mod-Severe Shigellosis Illness (Primary Endpoint)				
No. Severe Diarrhea only				
No. Moderate Diarrhea only				
No. Dysentery only				
No. Shigellosis Illness (Secondary Endpoints)				
No. Diarrhea only				
No. Dysentery only				
No. Fever only				
No. Satisfying the CVD historical definition of Severe Shigellosis				

Primary Endpoints, Definitions for Moderate-Severe Shigellosis Illness (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]
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 categories
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
‡ 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.

Table 7: Clinical & Bacteriological Responses to Challenge			
	Vaccine	**Placebo**	***P* value**
Median diarrheal stool volume, mL (IQR)			
Median duration of diarrheal stools, hr (IQR)			
Median number of diarrheal stools (IQR)			
Median of the maximum 24-hr loose stool output (IQR)			
No. any loose stools (%)			
No. any dysentery (%)			
No. any fever, ≥38.0°C (%)			
No. requiring early initiation of antibiotics (%)			
Median *Shigella* Disease Severity Score (IQR)			
Median peak *Shigella* count, cfu/mL (IQR)			
Median no. days of positive *Shigella* shedding (IQR)			

***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

Together with the efficacy read-out, results of the pro-inflammatory cytokine analysis during challenge (exploratory endpoint 1, D57-D64) will be reported. The results will be presented as a Figure and/or Table.

The interim report will also include preliminary immunogenicity data of anti-*Shigella flexn*eri 2a serum IgG up to the day of challenge. The analysis will include the samples from the 66 CHIM participants only.

Table 8 Anti-*S. flexneri* 2a serum IgG in CHIM participants at baseline and various timepoints up to the challenge

	anti-SF2a IgG TAU [units]		*If available*, **anti-SF2a IgG WRAIR [units]**	
	Vaccinees	Placebo recipients	Vaccinees	Placebo recipients
Day 1 (baseline)				
- GMT				
- 95% CI				
- Median				
- Frequency of responders				
- n				
Day 8				
- GMT				
- 95% CI				
- Median				
- Frequency of responders				
- n				
Day 29				
- GMT				
- 95% CI				
- Median				
- Frequency of responders				
- n				
Day 36				
- GMT				
- 95% CI				
- Median				
- Frequency of responders				
- n				
Day 56 (day before challenge)				
- GMT				
- 95% CI				
- Median				
- Frequency of responders				
- n				

G. Interim Analysis Report 2: Efficacy & Limited Immunogenicity

The Interim Analysis Report #2 will focus on the immunization and challenge phase of the study and provide all cumulative data to assess the primary and secondary endpoints of efficacy.

Efficacy assessment is completed with Day 66 and will be described completely in the analysis. The unblinded statistician will perform the efficacy analysis, as the data will continue to be blinded to the study team. The same efficacy reporting template from Interim Analysis Report 1 will be generated for this Interim Analysis Report 2.

Immunogenicity analyses will be included for all data points up to the day of challenge (D1 to D56 samples).

The Interim Analysis Report #2 will include the full analyses as described in the protocol for the following objectives and timeframes:

1. Primary endpoint analysis
    i. Per cohort (in case of slight variability of the freshly prepared challenge dose as this could influence the attack rate)
    ii. All volunteers combined
    iii. Summary table for determined challenge doses to be included.

2. Secondary endpoints analyses
    i. Safety and tolerability of 2 doses of SF2a-TT15 – **up to D56 (up to challenge)**
    ii. Performance of efficacy of SF2a-TT15 against different case definitions
    iii. Efficacy of SF2a-TT15 against fecal shedding of *S. flexneri* 2a
    iv. Serum IgG to SF2a LPS following vaccination – **up to D56 (up to challenge)**
        a) WRAIR analysis: 66 CHIM participants
        b) TAU analysis: i) CHIM participants only, ii) vaccinees only (who did not progress to the challenge), iii) all 90 volunteers combined
    v. Serum bactericidal activity following vaccination – **up to D56 (up to challenge)**
    vi. IgG and IgA ASC and ALS after vaccination – **up to D56 (up to challenge)**

3. Exploratory endpoints analysis:
    i. Pro-inflammatory cytokines in stool following challenge – **D57 to D64 (during challenge)**
    ii. IgG subclasses (analyzed only if efficacy was confirmed in the preliminary efficacy readout. Can be included here up to D56, up to challenge, if already available or in the complete final report)
    iii. Serum IgA following immunization – **up to D56 (up to challenge)**
    iv. Urine sIgA following immunization – **up to D56 (up to challenge)**
    v. IgG and IgA ASC expressing homing markers – following vaccination – **up to D56 (up to challenge)**
    vi. Correlates of protection
        a) All data
        b) Per cohort? – based on results for the primary endpoint
    vii. Compare IgG results of the different institutes – **up to D56 (up to challenge)**

H. Final Analysis Reporting

A final report upon the completion of all planned immunogenicity analysis. The full analysis as described in the protocol will be performed. The data tables that were generated for the Safety Reports and Interim Analysis Reports may be revised and updated as part of inclusion in the Final Analysis Report. The Final Analysis Report is intended to be performed on the final locked database and unblinded.

Immunogenicity analyses for the report will include only samples from the 66 CHIM participants, unless otherwise stated and briefly summarized in the following. Serum IgG and IgA will be measured by TAU also in samples from the 24 volunteers who will receive vaccine or placebo but will not be challenged. ASC homing studies will be performed in 6 subjects per cohort.