NAVAL MEDICAL RESEARCH CENTER
INFECTIOUS DISEASE DIRECTORATE
ENTERIC DISEASES DEPARTMENT

Statistical Analysis Plan

"A Phase 1 Dose Escalating Study of a Prototype CS6 Subunit Vaccine with a Modified Heat-labile Enterotoxin from Enterotoxigenic *Escherichia coli* (ETEC)"

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1 Abbreviations and Definitions

AE	Adverse Event
CF	Colonization Factors
CRF	Case Report Form
dmLT	Double Mutant Heat-Labile Toxin
ETEC	Enterotoxigenic E. coli
ID	Intradermal
LT	Heat Labile Enterotoxin
SAP	Statistical Analysis Plan
ST	Heat Stabile Enterotoxin
Tukey's HSD	Tukey's HSD (Honestly Significant Difference) test

2 Introduction

Worldwide, diarrhea causes approximately 1.7 billion cases annually [1]. In developing countries, there is recognition of the disease burden and in many cases, significant efforts have been made to improve sanitation, nutrition, and treatment management. However, diarrhea related complications still results in approximately 760,000 deaths in children annually, with the highest numbers in those younger than two years of life [1, 2]. The recognized need for more early effective countermeasures has driven a number of research agendas, including the development of enteric vaccines.

ETEC, one of several pathotypes of diarrheagenic *E. coli*, causes a secretory diarrhea that can range in presentation from mild discomfort to cholera-like purging. It is the most prevalent bacterial cause of childhood diarrhea in developing countries, and while the estimated number of ETEC episodes and deaths vary among researchers, in one estimate, ETEC was thought to cause 210 million cases of diarrhea and 380,000 deaths annually among infants and young children [3-7]. ETEC illness in the young has also been associated with growth faltering [8], and the repeated episodes caused by this infection are likely to lead to declines in both physical and cognitive development [9], which in turn are considered to have attendant macroeconomic consequences in countries and regions most heavily afflicted [10]. It is also the leading cause of travelers' diarrhea, etiologically implicated in 30-50% or more of cases [11-14], and this may be markedly underestimated due to the insensitivity of testing methods [15]. Its dual importance in global public health and military/travel medicine has galvanized policy makers in both sectors to develop a safe, effective ETEC vaccine, though such efforts remain under-resourced.

ETEC express adhesive fimbriae [also known as colonization factors (CFs)], surface-exposed polymeric protein appendages that plays a vital role in the initial step of ETEC pathogenesis. CFs mediate initial ETEC adherence to, and colonization of, the small intestine, after which ETEC secrete one or both of two enterotoxins that induce fluid and electrolyte secretion resulting in watery diarrhea. The two enterotoxins produced by ETEC are heat-stable enterotoxin (ST) and heat-labile enterotoxin (LT). CFs have long been a prime target for vaccine research and development. Their role as protective antigens has been substantiated by a number of studies in populations naturally exposed to ETEC diarrhea as well as volunteer studies of experimentally induced diarrhea, as has the role of LT enterotoxin [16-20]. Evidence for the preventive role of anti-CF immunity also derives from studies showing that bovine milk antibody product with high antibody titers against ETEC, and more specifically, purified colonization factor antigens, provided protection to humans in challenge studies [21, 22]. To date, more than twenty-two serologically distinct CFs have been identified.

CS6 is an atypical polymeric antigen that is highly prevalent among ETEC disease isolates from various geographic regions [20, 23, 24]. It is a heteropolymer composed of two structural subunits, CssA and CssB, in a ca. 1:1 ratio that has been confirmed with publication of the CssA and CssB crystal structures [25]. Recent reports provide evidence that CS6 binds to the human intestinal cell lines Caco-2 and INT407 [26, 27], consistent with the adhesive role that CS6 is presumed to play in ETEC disease pathogenesis. Jansson et al. reported that purified CS6 and recombinant CssB fused to glutathione-S-transferase (GST) were both shown to bind to intestinal glycosphingolipid sulfatide by thin layer chromatography [28].

Given the relatively high global prevalence of CS6-ETEC, we view the development of a protective, recombinant derivative of CS6 as critical to our overall strategy to develop a broadly protective multivalent ETEC adhesin subunit vaccine. As such, with the current data known, development of the prototype CS6 adhesin-based vaccine began with the development and characterization of *in cis* donor strand complemented variants of CssA (dsc16CssA[His]6, referred to as dscCssA) and CssB (dsc16CssB[His]6, referred to as dscCssB). Based on multiple lines of evidence, ntd14dsc16BCssBA (a variant of the original dscCssBA in which the N-terminal 14 amino acids has been removed and a heterologous CssB-derived donor strand is used to complement the C-terminal CssA), here after termed CssBA, was selected as the lead vaccine prototype. This product was assessed serially in a mouse immunogenicity model and an *A. nancymaae* NHP vaccination-challenge model. We then scaled up fermentation and purification processes, in preparation for bioproduction under cGMP conditions. Subsequent mouse immunogenicity studies of the cGMP CssBA lot demonstrated similar anti-CS6 IgG response to that of the research grade lot.

3 Analytical Methods

For nominal outcomes, parameter estimates will be calculated with 95% confidence intervals utilizing valid asymptomatic and/or exact binomial estimates. For continuous outcomes, measures of central tendency will be estimated based on the data distribution. Data following a Gaussian distribution (or data for which the central limit theorem appear applicable) will be summarized across study groups using a mean and standard deviation (and/or 95% confidence intervals). Continuous data which are not normally distributed (or for which the central limit theorem is not applicable) and ordinal data will be summarized across study groups using a median and interquartile range. Data transformations will be applied to approximate a Gaussian distribution as applicable. In particular, titers will be log-transformed prior to statistical comparisons and $\log_{10}$-transformed means (and standard deviations) and/or geometric mean titers will be estimated.

In general, between-cohort comparisons will be examined with nonparametric tests (Kruskal-Wallis for continuous data and Fisher's exact test for categorical data) unless assumptions are fulfilled for Analysis of Variance (ANOVA) or Pearson's χ^2.

Importantly, this study is designed to enable estimates of clinical, immunological, and microbiological endpoints not to compare parameter estimates across study cohorts. Nonetheless, these comparisons across cohorts will be made and guided by an Omnibus null hypothesis as follows (where θ is any nominal, continuous or ordinal parameter): $\theta_{\text{Cohort A}}=\theta_{\text{Cohort B}}=\theta_{\text{Cohort C}}=\theta_{\text{Cohort D}}$

Post-hoc comparisons will include the following: Bonferonni, Sidak, Tukey's HSD or Newman-Keuls.

Additionally, nonparametric paired *t* tests (Wilcoxon paired signed rank test) may be used to compare continuous data at two separate time points unless assumptions are fulfilled for paired t-test. Additional

comparisons may include repeated measures analysis of variance with study group as the between subject factor and sample collection time-points as the repeated factor.

All statistical tests will be interpreted in a two-tailed fashion using an *alpha* = 0.05.

4 Study Objectives

4.1 Primary Objectives

- Evaluate the safety of CssBA ± dmLT given by IM injection

4.2 Secondary Objectives

- Evaluate immune responses following IM vaccination with CssBA ±dmLT
- Identify a safe and immunogenic dose and route of a CssBA-based vaccine to be used in a subsequent vaccine and experimental challenge trial

These exploratory objectives are beyond the scope of the SAP.

5 Study Methods

This is an open-label Phase 1 clinical trial of CssBA±dmLT in which a total of 50 subjects will receive three vaccinations via IM injection on days 1, 22, and 43 (See Table 2). Dose escalation of CssBA from 5µg to 15µg to 45µg, and dmLT from 100 to 500ng will take place as outlined below. Group A is considered a pilot group in which CssBA and dmLT will be administered separately. All 3 doses will be administered and subjects monitored for safety 7 days after the third vaccination, prior to the enrollment of subjects in Group B.

The decision to advance to the next group will be based solely on the safety assessment. A dose level with no occurrence of stopping criteria will prompt moving to the next higher level. All safety data will be summarized and reviewed with the SRC prior to advancing to dose escalation.

Approximately one week after the first group (Group A) receives the third vaccination dose (Day 49), an interim Safety Report will be prepared by the PI and Study Statistician for review by the SRC. The content of the report will be agreed upon by the PI and the SRC and will include, but not be limited to, all adverse events (solicited, unsolicited, expected and unexpected) as well as relevant safety endpoints. Advancement to Group B will be based entirely on this safety assessment. The SRC's concurrence to advance to the next group will be made and provided in written format. This process will be repeated after groups B, C, and D before enrollment for the next group.

dmLT will be administered at the 500 ng dose in groups D and E if no significant reactogenicity is observed in Group C.

Table 2. Study Design of Phase 1 Clinical Trial of Intramuscularly Administered CssBA with dmLT (N=50)

Group	N	Route	CssBA (µg)	dmLT (ng)
A	5	IM	5	0
	5	IM	0	100
B	10	IM	5	100
C	10	IM	5	500
D*	10	IM	15	100/500
E*	10	IM	45	100/500

*Plan to proceed with 500ng dose; however, if there is an aberrant safety signal in Group C, will proceed with the 100 ng dose (presuming no prior signal in Group B)

5.1 Inclusion-Exclusion Criteria

5.1.1 Inclusion Criteria

Subjects must meet all of the following criteria to be included in the study:

1. Healthy, adult, male or female, age 18 to 45 years (inclusive) at the time of enrollment
2. Completion and review of comprehension test (achieved $\geq$ 70% accuracy).
3. Signed informed consent document
4. Available for the required follow-up period and scheduled clinic visits.
5. Women: Negative pregnancy test with understanding (through informed consent process) to not become pregnant during the study or within three (3) months following last vaccination

5.1.2 Exclusion Criteria

General health criteria

1. Health problems (for example, intercurrent febrile illness, chronic medical conditions such as psychiatric conditions, diabetes mellitus, hypertension or any other conditions that might place the subject at increased risk of adverse events-study clinicians, in consultation with the PI, will use clinical judgment on a case-by-case basis to assess safety risks under this criterion. The PI will consult with the Research Monitor as appropriate.
2. Clinically significant abnormalities on physical examination
3. Immunosuppressive drugs (use of systemic corticosteroids or chemotherapeutics that may influence antibody development) or illness (including IgA deficiency, defined by serum IgA <7 mg/dL).
4. Women who are pregnant or planning to become pregnant during the study period plus 3 months beyond the last vaccination and currently nursing women

5. Participation in research involving another investigational product (defined as receipt of investigational product or exposure to invasive investigational device) 30 days before planned date of first vaccination or anytime through the last study safety visit.
6. Positive blood test for HBsAg, HCV, HIV-1/2
7. Clinically significant abnormalities on basic laboratory screening

Research Specific

1. Exclusionary skin history/findings that would confound assessment or prevent appropriate local monitoring of AEs, or possibly increase the risk of an AE.
2. History of chronic skin disease (clinician judgment)
3. Acute skin infection/eruptions on the upper arms including fungal infections, severe acne or active contact dermatitis
4. Allergies that may increase the risk of AEs
5. Regular use (weekly or more often) of antidiarrheal, anti-constipation, or antacid therapy
6. Abnormal stool pattern (fewer than 3 stools per week or more than 3 stools per day) on a regular basis; loose or liquid stools on other than an occasional basis

Prior exposure to ETEC or Vibrio cholerae

1. History of microbiologically confirmed ETEC or cholera infection in the last 3 years
2. Travel to countries where ETEC or *V. cholerae* or other enteric infections are endemic (most of the developing world) within 3 years prior to dosing (clinician judgment)
3. Symptoms consistent with Travelers' Diarrhea concurrent with travel to countries where ETEC infection is endemic (most of the developing world) within 3 years prior to dosing, OR planned travel to endemic countries during the length of the study
4. Vaccination for or ingestion of ETEC, cholera, or *E. coli* heat labile toxin within 3 years prior to dosing
5. Occupation involving handling of ETEC or *V. cholerae* currently, or in the past 3 years

6 Sample Size

The sample size for this study is limited by the early stage of the product concept/testing and is designed to evaluate preliminary safety data but not designed to show statistically significant differences between groups. Given the small number of subjects per group, the precision of our estimate for adverse events is limited. For example, using binomial probability formulae for no observed adverse events within the 8 subjects yields a 95% exact confidence interval of 0-31%. Follow-on studies evaluating seemingly safe and immunogenic doses will be required with larger numbers of subjects in order to better define the safety profile.

6.1 Purpose of the analyses

The primary study objective is to evaluate the safety of CssBA ± dmLT given by IM injection

Analysis Populations

6.1.1 Safety Population

All subjects that receive one or more doses of the investigational product(s) will be included in the safety analysis. Adverse events will be listed individually and summarized by body system and preferred terms within a body system for each treatment group. Serious and/or unexpected AEs will also be discussed on a case-by-case basis.

6.1.2 Immunology Populations

Analyses will include both qualitative (responder rates) and quantitative results (log_{10} transformed values). All subjects that receive at least 2 doses of the investigational product and have requisite post-vaccination samples collected will be included in the analysis.

6.2 Missing Data

All subjects selected to participate in the study and meet the necessary population requirements will be included in the safety and/or immunology analysis. Data will be assumed to be missing at random and missing data points will be excluded from analysis.

6.3 Confidence Intervals and p-values

Rates of all AEs will be summarized with point estimate for percentage and 95% confidence intervals. Immunological outcomes will also be summarized in a tabular format and graphed to demonstrate kinetics of response. Qualitative (responder rates) and quantitative assessments (log transformed values) will be analyzed. Median increases (fold rises) of antibody concentrations and seroconversion rates will be calculated along with their 95% confidence intervals. Geometric mean titers will also be determined and presented with their 95% confidence intervals. All statistical tests will be interpreted in a two-tailed fashion using an *alpha* = 0.05.

7 General Considerations

7.1 Analysis Population

7.1.1 Safety Analyses

All AE's will be assessed for severity by the investigator. Essential in this assessment is the medical and clinical considerations of all information surrounding the event including any medical interventions required. Each event will be assigned one of the following categories: mild, moderate, severe, or life-threatening. The criteria below may be used for any symptom not included in the grading scale.

The eCRFs for AEs will reflect only the highest severity for continuous days and event occurred.

Mild	Grade 1	Does not interfere with routine activities; minimal level of discomfort
Moderate	Grade 2	Interferes with routine activities; moderate level of discomfort
Severe	Grade 3	Unable to perform routine activities; significant level of discomfort
Potentially life-threatening	Grade 4	Hospitalization or ER visit for potentially life-threatening event

FDA guidelines for toxicity will be followed; however if a subject is evaluated in an emergency room for nonlife threatening illness or symptoms (ie, visits emergency department on weekend for mild

problems because the physician's office is closed), the information from that visit will be reviewed and severity of the adverse event will be assessed according to the subject's clinical signs and symptoms.

As defined by the ICH guideline for GCP, the term "severe" is often used to describe intensity (severity) of a specific event (as in mild, moderate, or severe myocardial infarction); the event itself however, may be of relatively minor medical significance (such as severe headache). This is not the same as "serious," which is based on subject/event outcome or action criteria usually associated with events that post a threat to the subject's life or functioning. Seriousness (not severity) serves as a guide for defining regulatory obligations.

The proportion of all subjects with each AE will be summarized with point estimate for percent and 95% confidence intervals calculated using valid asymptomatic and/or exact binomial estimates. Summary tables will be created which will describe the number and percentage of subjects who experience each adverse event. In addition, tables will be prepared to list each adverse event, the number of subjects experiencing an event at least once, and the proportion of subjects with adverse event(s). Adverse events will be divided into defined severity grades (mild, moderate, severe, or potentially life-threatening). The tables will also divide the adverse events by severity and related (definite, probable, possible) or unrelated (unlikely, not related) to the investigational product.

All subjects who receive vaccination will be included in the safety analysis. Adverse event data will be listed individually and summarized by body system and preferred terms within a body system for each treatment group. Serious and/or unexpected AEs will also be discussed on a case-by-case basis. For the tabulation of the AEs by body system, a subject will be counted only once in a given body system. For example, a subject reporting nausea and diarrhea will be reported as one subject, but the symptoms will be listed as two separate AEs within the class. Therefore the total number of AEs reported within a body system may exceed the number of subjects within the body system reporting AEs.

Rates of all adverse events will be analyzed by Pearson's Chi-square test (or Fisher's exact test if assumptions are not met for Pearson's Chi-square) to compare dose levels. Summary tables will be created which will indicate the number of subjects who experienced events. Vaccine-related events (probably or possibly related) will be tabulated by study group. In addition, tables will be prepared to list each adverse event, the number of subjects in each treatment group who experienced an event at least once, and the rate of subjects with adverse event(s). Adverse events will be divided into defined severity grades (mild, moderate, severe). The tables will also divide the adverse events by severity and relationship to the investigational product. All immunized subjects will be included in the safety analysis.

8 Immunology Analyses

Analyses will include both qualitative (responder rates) and quantitative outcomes.

Graphical displays of immune responses will include the following:

1) Serum IgG and IgA responses to LT and CS6
2) Antibody Lymphocyte Supernatants (ALS) responses to LT and CS6

Between-group comparisons will be examined with nonparametric tests (Kruskal-Wallis for continuous data and Fisher's exact test for categorical data) unless assumptions are fulfilled

for Analysis of Variance (ANOVA) or Pearson's χ^2. Nonparametric paired *t* tests (Wilcoxon paired signed rank test) will be used to compare individual post-challenge to pre-challenge response within each treatment group unless assumptions are fulfilled for paired t-test. Comparisons of ALS responses post-vaccination will be performed using the Kruskal-Wallis test. All statistical tests will be interpreted in a two-tailed fashion using $\alpha = 0.05$. Statistical analyses will be performed using SAS v9.x for Windows (The SAS Institute, Cary, NC).

Immunologic Responder Definitions

Serology: Serum samples will be assayed for antibody IgG and IgA titers against LT and CS6 using ELISA methods previously established in the NMRC Immunology Laboratory. Previously established high-titer specimens will be included on each plate to track day to day interassay variation. For each antigen, pre- and post-vaccination serum samples will be assayed concurrently. The antibody titer assigned to each sample will represent the geometric mean of duplicate tests performed on two different days. Seroconversion will be defined as a $\geq$ 4-fold increase in endpoint titer between pre- and post- vaccination samples and a post-vaccination reciprocal titer greater than 10. A 4-fold rise is calculated by dividing the post-challenge reciprocal endpoint titer by the day pre-challenge reciprocal endpoint titer. Once a subject is defined as 'immunologic responder', that person is permanently categorized as a 'RESPONDER'. All statistical analyses will be performed on $\log_{10}$ – transformed titer values. Titers will be displayed graphically as $\log_{10}$ reciprocal endpoint titers or geometric mean titers. For subjects with a reciprocal titer less than 5, they will be assigned a value of 2.5 for computational purposes.

Antibody in Lymphocyte Supernatants (ALS): Peripheral Blood Mononuclear Cells (PBMCs) will be collected to determine antibody responses from Lymphocyte Supernatant against CS6 and LT. Antibody in Lymphocyte Supernatant (ALS) is an indirect quantification of antibody secreting cells (ASC) activated in the mucosa that circulate in the peripheral blood about seven days post-mucosal immunization/infection. This method has been shown to be a replacement for ELISPOT methodology. PBMCs are incubated without stimulation and the supernatant is later assayed for antigen-specific IgG and IgA Abs by ELISA. Seroconversion will be defined as a > 4-fold increase in endpoint titer between pre- and post- challenge samples. A 4-fold rise is calculated by dividing the post vaccination reciprocal endpoint titer by the pre-vaccination reciprocal endpoint titer. Once a subject is defined as 'immunologic responder', that person is permanently categorized as a 'RESPONDER'. All statistical analyses will be performed on $\log_{10}$ – transformed titer values. Titers will be displayed graphically as $\log_{10}$ reciprocal endpoint titers or geometric mean titers.

Exploratory Endpoints and Responder Definitions

Stool and saliva samples will be collected to explore the antigen-specific IgA response at the mucosal level by measuring IgA antibody titers against CS6 and LT. Immunologic responders will be defined as subjects with a > two-fold increase in reciprocal endpoint titer.

Saliva IgA: Total IgA content in the saliva extract samples will be determined by a modified ELISA method using commercial purified total IgA standard. Specimens with IgA concentration < 10 μg/ml will be excluded from further analysis, since antibody

titrations of specimens with such low IgA content give unreliable results. Subsequently, no comparison to post-immunization values will be performed. Specific antibody levels in the salivary extracts will be determined using similar ELISA methods described above. Salivary antibodies will be reported as adjusted end-point titers. Adjusted end-point titers will be calculated by dividing the antigen specific reciprocal titer value by the Total IgA reciprocal titer value. A ≥ 4-fold increase in the specific IgA per total IgA content between pre- and any post-vaccination specimens is considered a responder. All statistical analyses will be performed on $\log_{10}$ – transformed titer values.

Fecal IgA: Stool samples will be collected by subjects to assess fecal IgA immune responses. Total IgA content in the fecal extract samples will be determined by a modified ELISA method using commercial purified total IgA standard. Specific antibody levels in the fecal extracts will be determined using similar ELISA methods described above. Fecal antibodies will be reported as adjusted end-point titers. A ≥ 4-fold increase in the specific IgA per total IgA content between pre- and any post-vaccination specimens is considered a responder. All statistical analyses will be performed on $\log_{10}$ – transformed titer values.

9 Figures

Sample data tables and figures are included below to guide through the analysis and data presentation. Final tables and figures may be modified to optimize data presentation.

Results

Table 3: Demographic characteristics of study subjects

Characteristic	Participant (n =)	Screened (Not Enrolled)
Mean Age (sd)*	()	()
Age range	-	-
Gender (%)		
Male	()	()
Female	()	()
Race/Ethnicity		
African-American	()	()
Caucasian	()	()
Asian-American	()	()
Other	()	()

*Measured in mean (standard deviation)

Table 4: Baseline characteristics of study participants (by cohort)

Cohort	A		B	C	D	E
	A-1	A-2				
Age[a]						
Gender [N (%)]						
Male						
Female						
Race/Ethnicity [N (%)]						
African-American						
White						
Asian						
Other						
LT IgA						
LT IgG						
CS6 IgA						
CS6 IgG						

[a] Presented as mean (standard deviation) or median (interquartile range)

Table 5. Safety profile of all adverse symptoms coded as 'related' to vaccine following vaccination [n(%)] (Groups compared using a Chi-squared analysis)

AE	**Cohort A**			**Cohort B**			**Cohort C**			**Cohort D**			**Cohort E**		
Dose	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**
AE1															
AE2															
AE#															

Table 6. Safety profile of all adverse symptoms coded as 'unrelated' to vaccine following vaccination [n(%)] (Groups compared using a Chi-squared analysis)

AE	**Cohort A**			**Cohort B**			**Cohort C**			**Cohort D**			**Cohort E**		
Dose	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**
AE1															
AE2															
AE#															

Immunology

Table 7: Serological Immunological Responses to LT and CS6

Cohort		LT				CS6			
		ALS		Serum		ALS		Serum	
		IgG	IgA	IgG	IgA	IgG	IgA	IgG	IgA
A	A-1								
	A-2								
B									
C									
D									
E									

Table 8: Saliva and Fecal Immunological Responses to LT and CS6

Group		Number of Volunteers (%)			
		LT		CS6	
		Salivary IgA	Fecal IgA	Salivary IgA	Fecal IgA
A	A-1				
	A-2				
B					
C					
D					
E					

10 Safety Review Report

Approximately one week after the groups A, B,C, and D receive the third vaccination dose (stud day 49), an interim Safety Report will be prepared by the PI and Study Statistician for review by the Safety Review Committee (SRC). The PI and the SRC will agree upon the content of the report and will include, but not be limited to, all adverse events (solicited, unsolicited, expected and unexpected) as well as relevant safety endpoints. An excerpt of the report showing the tables that will be included are copied below. The SRC will interpret the data included in the report to determine if the dosing was safe and if the study should continue to the higher dose. These analyses will not affect the end of study analysis.

INTERIM DOSE LEVEL SAFETY REPORT

Vaccine Cohort (Circle one)

A-1 (5 µg CssBA)
A-2 (100 ng dmLT)
B (5 µg CssBA + 100 ng dmLT)
C (5 µg CssBA + 500 ng dmLT)
D (15 µg CssBA + 100 or 500 ng dmLT)
E (45 µg CssBA +100 or 500 ng dmLT)

Vaccine Dose (Circle one) **1 2 3**

N =

Cumulative summary of adverse signs and symptoms								
Symptoms	**N (%) of volunteers exhibiting each adverse event**							
	Grade 1		**Grade 2**		**Grade 3**		**Grade 4**	
	R	**NR**	**R**	**NR**	**R**	**NR**	**R**	**NR**
Local site pain								
Local site pruritis								
Vaccine site rash/eruption								
Vaccine Site Swelling (As reported by subject)								
Vaccine Site Tenderness								
Fever (subjective or objective)								
Headache								
Loose stools								
Arthralgia								
Myalgia								
Malaise								

R: Any sign or symptom coded as at least possibly related to the study product.
NR: Any sign or symptom coded as unrelated to the study product.
Note: The highest severity of an adverse event recorded for each subject (by relationship strata).

COMBINED INTERIM SAFETY REPORT FOR 3-DOSE SERIES:

Vaccine Cohort (Circle one)

A-1 (5 µg CssBA)
A-2 (100 ng dmLT)
B (5 µg CssBA + 100 ng dmLT)
C (5 µg CssBA + 500 ng dmLT)
D (15 µg CssBA + 100 or 500 ng dmLT)
E (45 µg CssBA +100 or 500 ng dmLT)

N =

Cumulative summary of adverse signs and symptoms								
Symptoms	**N (%) of volunteers exhibiting each adverse event**							
	Grade 1		**Grade 2**		**Grade 3**		**Grade 4**	
	R	**NR**	**R**	**NR**	**R**	**NR**	**R**	**NR**
Local site pain								
Local site pruritis								
Vaccine site rash/eruption								
Vaccine Site Swelling (As reported by subject)								
Vaccine Site Tenderness								
Fever (subjective or objective)								
Headache								
Loose stools								
Arthralgia								
Myalgia								
Malaise								

R: Any sign or symptom coded as at least possibly related to the study product.
NR: Any sign or symptom coded as unrelated to the study product.
Note: The highest severity of an adverse event recorded for each subject (by relationship strata).

11 Reporting Conventions

P-values ≥0.001 will be reported to 3 decimal places; p-values less than 0.001 will be reported as "<0.001". The mean, standard deviation, and any other statistics other than quantiles, will be reported to one decimal place greater than the original data. Quantiles, such as median, or minimum and maximum will use the same number of decimal places as the original data. Estimated parameters, not on the same scale as raw observations (e.g. regression coefficients) will be reported to 3 significant figures.

12 Technical Details

Statistical analyses will be performed using SAS v9.x for Windows (The SAS Institute, Cary, NC).

13 References

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