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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Sponsor	PATH Vaccine Solutions (PVS) 455 Massachusetts Ave NW Suite 1000 Washington, DC 20001
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Clinical Trial Registration #	NCT03404674
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INVESTIGATOR'S AGREEMENT

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

"I have read this protocol and agree to conduct the study as outlined herein in accordance with International Council on Harmonisation Good Clinical Practice Guideline and FDA, DoD Regulations."

_____ _____

Signature of Investigator Date

Tida K. Lee, MD, PhD
Principal Investigator
Enteric Diseases Department
Naval Medical Research Center

PROCEDURES IN CASE OF EMERGENCY

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2. SYNOPSIS

Name of Sponsor: PATH Vaccine Solutions (PVS)

Name of Investigational Products:

- spd_dsc$_{16B}$ntd$_{14}$CssBA$_{B7A}$[His]$_6$ (CssBA) - recombinant ETEC CssBA protein
- LTR192G/L211A (dmLT) – *E. coli* double mutant heat labile toxin

Title of Study: A Phase 1 Dose Escalating Study of a prototype CS6 subunit vaccine with a Modified Heat-labile Enterotoxin from Enterotoxigenic *Escherichia coli* (ETEC)

Study center: Walter Reed Army Institute of Research (WRAIR) Clinical Trials Center (CTC)

Principal Investigator: Tida K. Lee, MD, PhD

Sub investigators: Ramiro Gutierrez, MD, MPH; Christopher Duplessis, MD, MPH, MS; Chad Porter, PhD, MPH; Milton Maciel, PhD; Paige Waterman, MD; Alison Lane, MD, MS; Mark Riddle, MD, DrPH; Melinda Hamer, MD, MPH

Study Duration (per group):	**Phase of development:** 1
Screening (up to 90 days); Vaccination to final blood draw (70 days); long term follow-up (12 months); immunology analysis (12 weeks); data analysis and report writing (2 months).	

Trial Objectives:

Primary Objective:

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

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 vaccination/challenge trial

Study Design and Methodology:

This is an open-label, Phase 1 clinical trial in which a total of 50 subjects will receive three injections of either CssBA alone, dmLT alone or CssBA + dmLT. The vaccine will be administered via IM injection to alternating deltoid regions on days 1, 22 and 43. Each subject will receive the same dose at each vaccination dependent upon group assignment. Group A is considered a pilot group in which 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.

Each subject will receive 3 doses and all subjects within a group will be enrolled on the same day. Progression to subsequent groups will be dependent on safety measured in the first 7 days after the third vaccination of the previous group.

Study Design for a Phase 1 clinical trial of intramuscularly administered CssBA with/without 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)

Blood, saliva, and stool specimens will be collected at prescribed intervals to examine systemic and mucosal immune responses. Vaccine safety will be actively monitored during vaccination and for 28 days following the third vaccine dose. The decision to advance to the next group is based solely on the safety assessment through 7 days after the subjects in the prior group receive the third vaccination. All safety data will be summarized and reviewed by the Safety Review Committee (SRC) prior to dose-escalation.

Estimated Number of Subjects Screened:

Based on prior studies with similar study designs, sample collections and inclusion/exclusion criteria, we anticipate screening approximately 5 subjects for every one subject that is enrolled in the study. Therefore we anticipate approximately 250 individuals will be screened.

Maximum Number of Subjects Enrolled:

A maximum of 50 subjects are planned for this study. Up to four alternates per group will be selected as well that may be enrolled in the event a subject becomes ineligible prior to receipt of the investigational product.

Main Criteria for Inclusion/Exclusion:

Inclusion Criteria:

- Healthy, adult, male or female, age 18 to 45 years (inclusive) at the time of enrollment.
- Completion and review of comprehension test (achieved $\geq$ 70% accuracy).
- Signed informed consent document.
- Available for the required follow-up period and scheduled clinic visits.
- 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.

Exclusion Criteria:

General Health

- Health problems (for example, intercurrent febrile illness, chronic medical conditions such as psychiatric conditions, diabetes mellitus, hypertension or any other condition 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.
- Clinically significant abnormalities on physical examination.
- Immunosuppressive drugs (use of systemic corticosteroids or chemotherapeutics that may influence antibody development) or illness (including IgA deficiency, defined by serum IgA <7mg/dL).
- Women who are pregnant or planning to become pregnant during the study period plus 3 months beyond the last received dose and currently nursing women.
- 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.
- Positive blood test for HBsAg, HCV, HIV-1/2.
- Clinically significant abnormalities on basic laboratory screening.

Research Specific

- Exclusionary skin disease history/findings that would confound assessment or prevent appropriate local monitoring of AEs, or possibly increase the risk of a local AE.
- History of chronic skin disease (clinician judgment).
- Acute skin infection/eruptions on the upper arms including fungal infections, severe acne or active contact dermatitis.
- Allergies that may increase the risk of AEs.
- Regular use (weekly or more often) of antidiarrheal, anti-constipation, or antacid therapy.
- 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*

- History of microbiologically confirmed ETEC or cholera infection in the last 3 years
- 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).
- 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.
- Vaccination for or ingestion of ETEC, cholera, or *E. coli* heat labile toxin within 3 years prior to dosing.
- Occupation involving handling of ETEC or *V. cholerae* currently, or in the past 3 years.

Investigational product dosages and modes of administration:

The investigational products are CssBA and dmLT. The initial group will assess the safety of the products independently with CssBA and dmLT alone.

Following the initial group, escalating doses of CssBA (5µg, 15 µg, 45 µg) will be given with escalating doses of dmLT (100 ng or 500ng). All subjects will receive 3 doses of the investigational product 3 weeks apart (study days 1, 22, and 43). The dose of dmLT in Groups D and E will be determined from the safety data from previous groups.

Duration of Treatment:

Subjects will receive 3 vaccine doses, each 3 weeks apart (study days 1, 22, and 43). In person follow up will continue for 28 days after receipt of the last vaccine dose.

Immunology:

Primary Immunogenicity:

- Serum samples will be assayed for antibody titers against LT and CS6.
- Peripheral Blood Mononuclear Cells (PBMCs) will also be collected to determine Antibodies from Lymphocyte Supernatant (ALS) responses to CS6 and LT.

Exploratory Immunogenicity:

- Saliva and stool samples will also be collected to assess for secretory IgA responses to CS6 and LT.
- Samples will be collected as outlined in the time and events schedule. In order to support additional future exploratory evaluations in systems biology, cells and serum samples will be collected for use in a variety of transcriptomic, proteomic, flow cytometry, memory B cells, and cytokine analysis. Any testing of samples for these exploratory outcomes will be performed under a modification to this protocol to further detail the assay(s) or performed under a separate research protocol.

Statistical methods:

With the early stage of the product concept/testing, the sample size for this study is designed to evaluate preliminary safety data but not designed to show statistically significant differences between groups. Initially, a small number of subjects (n=5) will receive CssBA and dmLT independently at the lowest tested doses. The small number of subjects in each of these groups is designed to minimize the risk of acute toxicity in this first-in-human study. Subsequent groups are planned with larger sizes (10 per group) for the purpose of safety and immunogenicity evaluation.

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 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, and 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.

For immune responses, both qualitative and quantitative (log10 transformed values for non-normally distributed samples) results will be analyzed. Geometric mean titers may be calculated along with their 95% confidence intervals. An immunological responder will be defined as those subjects with a ≥4 fold rise in reciprocal baseline titer. Between groups comparisons may be examined with nonparametric tests (Kruskal-Wallis for continuous data and Fisher's exact test for categorical data) unless assumptions are fulfilled for Student's t or $\chi 2$.

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. Only subjects who receive at least 2 vaccine doses will be included in the immunology analysis. All statistical tests will be interpreted in a two-tailed fashion using an alpha = 0.05 to represent statistical significance.

3. TABLE OF CONTENTS

4. LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

The following abbreviations and specialist terms are used in this study protocol.

Table 2: Abbreviations and Specialist Terms

Abbreviation or Specialist Term	Explanation
°C	degrees Celsius
Ab	antibody
Abs	antibodies
AEs	adverse event
Ag	antigen
ALS	antibodies from lymphocyte supernatant
ALT	alanine aminotransferase
ASC	antibody-secreting cell
AST	aspartate aminotransferase
B7A	Enterotoxigenic Escherichia coli strain expressing colonization factor CS6
BB-178B	research lot of CssBA
BP	blood pressure
BPR	batch production record
BUMED	United States Navy Bureau of Medicine and Surgery
BUN	blood urea nitrogen
CBC	complete blood count
CD4	T cell cluster of differentiation 4
CF	colonization factor
CFA	colonization factor antigen
CfaE	colonization factor antigen E
CfaEB	colonization factor antigen EB
CFR	Code of Federal Regulations
CFU	colony forming units
cGMP	current good manufacturing practices
Cl-	Chloride
CS3	Coli surface antigen 3
CS6	Coli surface antigen 6
CssA	Structural subunit of CS6; component of CssBA
CssB	Structural subunit of CS6; component of CssBA
CTC	Clinical Trials Center
dsc	donor strand complemented
ELISA	Enzyme-Linked Immunosorbent Assay
ELISPOT	Enzyme-Linked ImmunoSpot
ETEC	enterotoxigenic *Escherichia coli*
FBS	fetal bovine serum
FDA	Food and Drug Administration
g	gram

Abbreviation or Specialist Term	Explanation
G	gauge
GI	gastrointestinal
GLA	glucopyranosyl lipid adjuvant
GLA-AF	glucopyranosyl lipid adjuvant aqueous formulation
GLA-SE	glucopyranosyl lipid adjuvant stable emulsion
GLP	Good Laboratory Practice
GST	glutathione-S-transferase
HAI	hemagglutination inhibition
HBsAg	Hepatitis B surface antigen
HCO3-	bicarbonate
HCV	Hepatitis C virus
HIB	*Haemophilus influenzae* type B
HIV-1	human immunodeficiency virus 1
HIV-2	human immunodeficiency virus 2
HR	heart rate
ICH	International Council on Harmonisation
ID	Intradermal
IDRI	Infectious Disease Research Institute
IgG	immunoglobulin G
IgG1	Immunoglobulin G1
IgG2	Immunoglobulin G2
IgG3	Immunoglobulin G3
IM	Intramuscular
IN	Intranasal
IND	investigational new drug
IRB	Institutional Review Board
K	potassium
K+	potassium ion
kg	Kilogram
LT	*Escherichia coli* heat-labile enterotoxin
LT(R192G/L211A) (dmLT)	*Escherichia coli* double mutant heat labile toxin with two mutations at amino acids 192 and 211
MD	Maryland
MD2	myeloid differentiation factor 2
mg	milligram
Min	minutes
mL	milliliter
mLT (LTR192G)	*Escherichia coli* mutant heat labile toxin with a mutation at amino acid 192
MOP	Manual of Procedures
N	normal
Na	Sodium
N/A	not applicable

Abbreviation or Specialist Term	Explanation
ND	not done
NFkB	nuclear factor kappa-light-chain-enhancer of activated B cells
NHP	non-human primates
NMRC	Naval Medical Research Center
OSP	O-specific polysaccharide
PBMCs	peripheral blood mononuclear cells
PBS	phosphate-buffered saline
PI	Principal Investigator
PM	post meridiem (i.e., afternoon)
PVS	PATH Vaccine Solutions
rLTB	recombinant heat-labile toxin B subunit
SAEs	serious adverse events
SC	subcutaneous
SD	standard deviation
SLA-SE	second-generation glycopyranosyl lipid-A stable emulsion
SOP	standard operating procedure
spd	signal peptide
SRC	Safety Review Committee
ST	heat-stable enterotoxin
SUSAR	serious and unexpected adverse event
T	temperature
TC	transcutaneous
TCI	transcutaneous immunization
TD	travelers' diarrhea
TH1	T helper cell type 1
TLR4	toll-like receptor 4
TLR4-MD2	toll-like receptor 4-myeloid differentiation factor 2 complex
TLRs	toll-like receptors
WBC	while blood cell
WIRB	Western Institutional Review Board
WRAIR	Walter Reed Army Institute of Research
WRAIR PBF	Walter Reed Army Institute of Research Pilot Bioproduction Facility
β-hCG	Beta human chorionic gonadotropin
µg	microgram

5. INTRODUCTION

5.1. Background

5.1.1. Epidemiology

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 result 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 studies, 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.

5.1.2. CS6 Study Rationale

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 have 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.

5.2. Nonclinical studies

5.2.1. Mouse Study

5.2.1.1. Immunogenicity of CssBA in Mice

In Balb/C mice, CssBA (research grade) elicited strong IgG and IgA anti-CS6 serum titers after intradermal (ID) vaccination (admixed with mLT adjuvant), at levels that were similar or greater in magnitude compared to any of the other heterodimers. Three IN immunizations with 125 or 25 µg of CssBA elicited levels of serum anti-CS6 Abs at day 42 comparable to the ID immunization with the same protein at 10 µg (see Table 3). A dose dependent drop in the levels of serum anti-CS6 IgG and IgA Abs was observed when IN immunizations were performed with 2.5 and 0.25 µg of CssBA. Similar patterns were observed with sera collected on day 28, after two immunizations (data not shown).

Table 3. CssBA Mouse Immunogenicity Responses in Baseline Evaluation

Protein	Dose	Route	LT Component	Anti-CS6 IgG titer (Log_{10}) mean±SD	Anti-CS6 IgA titer (Log_{10}) mean±SD
CssBA	10 µg	ID	LTR192G (0.1 µg)	5.3±0.24	1.7±0.33
CssBA	125 µg	IN	LTR192G (1.5 µg)	5.5±0.19	4.1±0.4
	25 µg	IN	LTR192G (1.5 µg)	5.6±0.18	4.2±0.15
	2.5 µg	IN	LTR192G (1.5 µg)	4.7±0.15	3.1±0.92

	0.25 µg	IN	LTR192G (1.5 µg)	2.6±1.3	1.4±0

5.2.2. Nonhuman Primate Study

Following the mouse study, the vaccine (research grade) was tested in owl monkeys (*A. nancymaae)*. The monkeys were vaccinated intradermally with CssBA plus dmLT (100ng), CS6 plus dmLT or dmLT alone, while negative control monkeys were inoculated with PBS on days 0, 14, 28, and 84. There were eight monkeys per group. The products were dosed: CssBA 100µg, dmLT 100ng, and CS6 100µg. Animals vaccinated with CssBA or CS6 demonstrated high serum anti-CS6 IgA and IgG titers following the second vaccination, which remained high following the third and fourth vaccinations. All animals that received dmLT demonstrated high serum anti-dmLT IgA and IgG titers following the second vaccination. Following vaccination, the monkeys were challenged on study day 98 (two weeks after last vaccination dose) with a CS6 expressing, LT+ ETEC strain (B7A). Monkeys vaccinated with CssBA and dmLT demonstrated 100% protective efficacy, whereas vaccination with CS6 and dmLT provided 80% protective efficacy. Monkeys vaccinated with dmLT alone demonstrated a 40% protective efficacy, although this result was not statistically significant (see Table 4).

Table 4. Protective Efficacy of Intradermal Vaccination Group Compared to PBS Control (N = 8/group)

Group	Regimen	Diarrhea Attack Rate (%)	Protective Efficacy (%)	P-value
1	CS6 + dmLT	12.5	80	0.12
2	CssBA + dmLT	0	100	0.03
3	dmLT	37.5	40	0.62
4	PBS	62.5	-	-

5.2.3. Preclinical GLP Toxicology of CssBA and dmLT in Sprague-Dawley Rats

5.2.3.1. Brief Summary

A repeat dose GLP-compliant toxicology study was performed in Sprague-Dawley rats to assess the toxicity and local site reactogenicity of subunit ETEC vaccine candidates, CssBA and dmLT by IM injection. The study also included investigation of the ID route of administration and with addition of SLA-SE via IM injection, although the ID route and SLA-SE will not be evaluated in this protocol. This study was conducted in compliance with GLP at Sinclair Research in Auxvasse, Missouri.

5.2.3.2. Methods

One-hundred and twenty male and 120 female rats were randomized into eight study groups by stratified body weights as shown in Table 5.

Table 5. Preclinical Toxicology Study Design

Group	Dose Route	Test Article (Dose/Inj.)			Dose Vol. (mL/In)	No. of Animals			
						Main		Recovery	
		CssBA	dmLT	SLA-SE		M	F	M	F
1	ID	NA [a]	NA [a]	NA [a]	0.1	10	10	5	5
2	ID	45 µg	-	-	0.1	10	10	5	5
3	ID	45 µg	1 µg	-	0.1	10	10	5	5
4	IM	NA [a]	NA [a]	NA [a]	0.25	10	10	5	5
5	IM	45 µg	0.5 µg		0.25	10	10	5	5
6	IM	45 µg	0.5 µg	5ug	0.25	10	10	5	5
7	IM	45 µg	1 µg	-	0.25	10	10	5	5
8	IM	45 µg	1 µg	5 µg	0.25	10	10	5	5

Note: ID = Intradermal injection; IM = Intramuscular Injection;
[a] Saline Control Group

Animals were immunized a total of four times, by the ID or IM routes as shown in Table 5, with each dose administered three weeks apart on Study Days 1, 22, 43, and 64. Animals assigned to the main study cohort were sacrificed on Study Day 66, two days after the fourth and final vaccination. A second cohort of animals, designated as recovery, went through a 28-day treatment-free period prior to sacrifice on Study Day 92.

Standard toxicology endpoints were evaluated, including body weights, food consumption, body temperatures at 6 and 24 hours post-dose, ophthalmology, detailed physicals, and daily clinical observations. Injection sites were evaluated and scored, according to a modified Draize (1965) method, daily at 24, 48, and 72 hours for all animals following each dose. If a positive score (> 0) was observed for an individual animal at 72 hours post dose, that animal's dose site was evaluated daily until a score of zero for both erythema and edema was achieved. Injection sites were also evaluated daily as part of the clinical observations and included an evaluation of apparent pain and other signs of local tolerance or toxicity issues such as presence of a hematoma, ulceration, vesiculation, or eschar.

To avoid sampling effects on hematology parameters, animals were assigned for evaluation of hematology, coagulation, or clinical chemistry during the in-life phase on Study Day 3 (all parameters) and Study Day 7 (coagulation and chemistry only). All parameters were evaluated on all animals at baseline and at sacrifice. Immunology was conducted to evaluate anti-CS6 and anti-LT serum IgG at baseline and at sacrifice.

At each sacrifice the assigned animals underwent a full gross necropsy, including organ weights, and tissues were collected. Microscopic examination of all tissues was conducted on animals sacrificed on Study Day 66. Only injection sites and gross lesions (none present) were evaluated from animals sacrificed on Study Day 92.

5.2.3.3. Results

There were no significant effects on ophthalmology, body weight gains, food consumption, or body temperatures. Test article related changes in hematology, coagulation parameters, and clinical chemistry were observed, including increases in platelet count, fibrinogen, and increases of the globulin fraction of serum total protein. These findings were consistent with an acute inflammatory or immune-stimulatory effect of vaccination, short-lived, and non-adverse. Systemically, vaccination with CssBA and dmLT was well-tolerated and immunogenic. Test article related clinical findings were limited to the injection site.

IM co-administration of 45 µg of CssBA and 0.5 µg of dmLT was associated with moderate to severe edema, including a more regional limb swelling, following the first injection. At subsequent doses the severity of this finding was substantially reduced. Erythema was minimal to moderate at the injection site. The incidence and severity of dose-site in-life findings in animals treated with saline (Group 4) or 45 µg CssBA and 0.5 µg dmLT (Group 5) is summarized in Table 6.

Table 6: Dose Site Observations Following Vaccination

Dose Administration Day			**Dose Day 1**		**Dose Day 22**		**Dose Day 43**		**Dose Day 64**	
Group			**4**	**5**	**4**	**5**	**4**	**5**	**4**	**5**
CssBA (µg/mL)			**NA**	**45**	**NA**	**45**	**NA**	**45**	**NA**	**45**
dmLT (µg/mL)			**NA**	**0.5**	**NA**	**0.5**	**NA**	**0.5**	**NA**	**0.5**
N			**30**	**30**	**30**	**30**	**30**	**30**	**30**	**30**
Limb Swelling	**% Distribution of Maximum Magnitude**	**None**	100%	7%	100%	73%	100%	47%	90%	37%
		Slight	0%	40%	0%	27%	0%	43%	10%	23%
		Mod	0%	53%	0%	0%	0%	7%	0%	40%
		Sev	0%	0%	0%	0%	0%	3%	0%	0%
	Duration	**Mean**	0	3.03	0	0.37	0	1.2	0.1	0.87
		SD	0	1.35	0	0.67	0	1.3	0.31	0.78
Erythema	**% Distribution of Maximum Score**	**0**	100%	87%	100%	93%	100%	97%	100%	100%
		Min	0%	3%	0%	7%	0%	3%	0%	0%
		Mild	0%	3%	0%	0%	0%	0%	0%	0%
		Mod	0%	3%	0%	0%	0%	0%	0%	0%
		Sev	0%	3%	0%	0%	0%	0%	0%	0%
	Duration	**Mean**	0	0.13	0	0.07	0	0.07	0	0
		SD	0	0.35	0	0.25	0	0.37	0	0

Dose Administration Day			Dose Day 1		Dose Day 22		Dose Day 43		Dose Day 64	
Group			4	5	4	5	4	5	4	5
CssBA (µg/mL)			NA	45	NA	45	NA	45	NA	45
dmLT (µg/mL)			NA	0.5	NA	0.5	NA	0.5	NA	0.5
N			30	30	30	30	30	30	30	30
Edema	% Distribution of Maximum Score	0	100%	0%	100%	100%	100%	73%	90%	50%
		Min	0%	23%	0%	0%	0%	27%	10%	10%
		Mild	0%	53%	0%	0%	0%	0%	0%	37%
		Mod	0%	17%	0%	0%	0%	0%	0%	3%
		Sev	0%	7%	0%	0%	0%	0%	0%	0%
	Duration	Mean	0	2.83	0	0	0	0.27	0.1	0.53
		SD	0	1.46	0	0	0	0.45	0.31	0.57

Min = minimal; mod = moderate; sev = severe

No manifestations of gross toxicity at the injection site such as hematoma, vesiculation, or ulcers were observed at this dose and route.

At necropsy on Day 66, there were no gross observations noted for this treatment group nor differences in organ weights, either absolute or relative to body or brain weight. Microscopically, hyperplasia of the inguinal lymph node was observed in some animals, consistent with a post-vaccination response. Injection site observations included minimal to mild inflammation of skeletal muscle and subcutis at all four injection sites, although at a higher incidence in the left biceps femoris (second dose; Day 22) and left gluteus (fourth dose, Day 64). Following a 28-day recovery period, these findings had largely resolved and no chronic process was apparent. Overall, the vaccine induced an acute reactogenicity following the first dose, but was overall well-tolerated and non-adverse at 45 µg CssBA and 0.5 µg dmLT.

When the dose of dmLT was increased to 1 µg with 45 µg CssBA IM, the incidence and severity of swelling increased. There were also observations of apparent pain in some dosed animals following the first dose. Changes in clinical pathology parameters associated with inflammation such as fibrinogen, platelet increases, changes in clotting time, and increase in the globulin fraction were higher in this group, consistent with the dose site clinical findings. As a result, the clinical trial design was modified to escalate to the 0.5 µg dmLT dose level and not exceed that dose.

Addition of SLA-SE, regardless of the dose of dmLT (0.5 or 1 µg), via IM injection led to severe edema and limb swelling in 100% of vaccinated animals after the first dose. Animals in these groups (Groups 6 and 8) also exhibited apparent pain upon palpation of the dose site following the first dose; six animals were observed limping or favoring the injected limb and three animals were treated for pain with buprenorphine. Pain was also observed following the fourth dose. The incidence and severity overall decreased for these groups over the course of the study, but some severe edema and limb swelling was still observed after the fourth dose. Microscopically, minimal to marked inflammation of the skeletal muscle, subcutis, and/or dermis was observed at

the injection sites as well as hematoma, fibrin exudation, and subcutaneous edema. Severity and incidence was higher at dose sites 2 (left biceps femoris) and 4 (left gluteus), suggesting that the inflammatory response is more regional and related to the local draining lymph node. These findings are consistent with vascular damage related to reactogenicity. Due to the severity of local effects and microscopic findings, the clinical trial design was modified to not include SLA-SE adjuvant.

By the ID route, 45 µg CssBA was well-tolerated. Local site observations were similar to that seen in the saline ID route control animals (Group 1). Mild and transient changes in hematology, coagulation, and clinical chemistry were observed, consistent with an immune-stimulatory effect of vaccination. There were few test-article related findings microscopically, with just minimal residual inflammation observed at the injection site. Serum IgG anti-CS6 responses were low, even after four doses. All females, but not all males, seroconverted by Study Day 92.

When 1 µg of dmLT was co-administered with 45 µg CssBA intradermally, an adverse effect was observed. As the study progressed, dose site reactogenicity, specifically erythema, was substantially increased in all animals assigned to this treatment group. By the fourth and final dose 25 of 30 animals were observed with an eschar at the injection site and were determined to exhibit apparent pain upon palpation of the injection site. Hematoma was also observed in 21 of 30 animals and ulcers were observed in 7 of 30 animals. Microscopic evaluation revealed hematoma, inflammation of the dermis, subcutis, and underlying muscle, and fibrin exudation. These findings were especially pronounced at dose sites 2 (left biceps femoris) and 4 (left gluteus). These responses are consistent with reactogenicity. The clinical trial design was therefore modified to remove the ID route of administration.

5.2.3.4. Conclusions

In Sprague-Dawley rats, 45 µg CssBA and 0.5 µg dmLT by IM injection is well-tolerated and immunogenic. Mostly mild to moderate, short-lived edema and erythema as well as systemic inflammatory effects were observed, but non-adverse. This study supports the revised design of the Phase 1 study.

A higher dose of dmLT (1 µg) with 45 µg CssBA IM is associated with increased inflammatory responses in rats, thus is not supported by current animal studies. Addition of 5 µg SLA-SE to 45 µg CssBA and either 0.5 or 1 µg dmLT IM led to adverse effects such as limping and pain and an apparent immunotoxic reaction. Thus, a higher dose of dmLT and inclusion of SLA-SE are not supported by current animal toxicology data.

ID administration of 45 µg CssBA was well-tolerated, but poorly immunogenic. Addition of 1 µg of dmLT to 45 µg CssBA by ID injection led to substantial and escalating dose site reactogenicity and apparent immunotoxicity. This study supports the safe dosing of 45 µg CssBA by the ID route, but the inclusion of dmLT at any dose level is not supported.

5.3. dmLT

5.3.1. dmLT Clinical Experience

Given the likely importance of anti-LT responses in any successful ETEC vaccine, CssBA will be co-administered with dmLT. To that end, a series of phase 1 trials with the CFA/I adhesin prototype candidate have recently been completed (Riddle and Gutierrez et al, unpublished data,

NCT01382095, NCT01644565) in which the prototype vaccine was co-administered transcutaneously and intradermally with LTR192G, or mLT, an *Escherichia coli* mutant heat labile toxin with a mutation at amino acid 192. While 50 μg of LTR192G administered transcutaneously was safe and induced serum IgG and IgA responses in 75-100% of subjects, the magnitude of the responses was relatively low across all immune parameters. In contrast, anti-LT responses in subjects administered 100 ng of mLT intradermally was safe and demonstrated both a higher rate of serologic responses and ALS responses. Intradermal use of mLT was associated with transient mild to moderate induration, hyperpigmentation and erythema in all subjects that did not increase in intensity with subsequent doses. Local pruritus was also seen but somewhat less frequently, and mLT given alone at 100 ng gave similar local reactogenicity results to the mLT plus CFA/I adhesin prototype vaccine combination. Given the tolerable safety profile and immunogenicity results, the intradermal vaccine was moved into a Phase 2/2b challenge study in which a total of 56 subjects underwent vaccination and similar to the phase 1 trial there were no vaccine-attributable systemic adverse events while vaccine site reactions (rash) and pruritus were common. There is no previous human experience of dmLT given by the intradermal or intramuscular route; however, a first in man Phase 1 dose-escalation study evaluating the safety and tolerability of dmLT administered via the intradermal route is currently underway at CCHMC (DMID Protocol 13-0013; NCT02531685) and oral dmLT administered alone in a dose of up to 100 μg and together with an inactivated ETEC vaccine and live attenuated ETEC vaccine in a dose of up to 25 μg were all safe in their respective Phase 1 studies. Finally, comparative preclinical data in mice indicate that dmLT may be less reactogenic at the injection site than mLT.

5.4. Vaccine Administration

The investigational products will be administered intramuscularly. Ease and experience with IM vaccinations, without need for costly devices or additional training, would allow for more optimal transition to use in developing countries. Neither of these vaccine antigens has been administered intramuscularly so the anticipated immune response profile is uncertain.

The purpose of the study is to determine if immunization with CssBA with dmLT is safe and immunogenic. If the vaccines are safe and induce robust humoral and mucosal immune responses, a vaccination/challenge study may occur to assess efficacy in a robust experimental ETEC challenge model.

6. TRIAL OBJECTIVES

6.1. Primary Objective

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

6.2. 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

7. INVESTIGATIONAL PLAN

7.1. Study Design

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 7). 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. Following Groups A, B, C, and D, a safety analysis will be completed and reviewed by the Safety Review Committee (SRC). Progression to subsequent groups will be dependent on safety measured in the first 7 days after the third vaccination of the previous group.

Table 7. 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)

7.2. Number of Subjects

Based on prior studies with similar study designs, sample collections and inclusion/exclusion criteria, we anticipate screening approximately 5 subjects for every one subject that is enrolled in the study. Therefore we anticipate approximately 250 individuals will be screened. A maximum of 50 subjects are planned for this study. Up to four alternates per group will be selected as well that may be enrolled in the event a subject becomes ineligible prior to receipt of the investigational product.

7.3. Screening and Study Schedule

The screening visit is scheduled -90 to -2 days before the first vaccination. Fully informed, written consent must be obtained from each subject prior to conducting any study procedure.

Two consent forms will be signed; a consent form for study participation and a separate consent form for HIV testing. To assess understanding, a comprehension assessment will be administered after the subjects sign the study participation written consent. The following evaluations/ procedures will be carried out:

- Oral and/or video presentation of clinical trial design, risks and study schedule
- Review of informed consent document
- One-on-one discussions with principal investigator (PI) or sub investigator
- Signature of informed consent document
- Comprehension test (minimum of 70% accuracy required for participation)
- Medical history – between Days - 90 to -2
- Physical examination – between Days -90 to -2
- Vital Signs – between Days -90 to -2
- Screening laboratory analysis (allowable time period for accepting test):
  - Complete blood count (between Days -90 to -2)
  - Serum transaminases (ALT/AST) (between Days -90 to -2)
  - Na, K+, CL-, HCO_3, glucose, BUN and creatinine (between Days -90 to -2)
  - Pregnancy test (urine -hCG) for women, as well as 0-24 hours before each vaccination
  - Total serum IgA (between Days -90 to -2)

Additionally, approximately 7 days prior to vaccination (allowable range: Days -14 to -2), subjects will have a follow-up medical history and brief physical exam to ensure ongoing eligibility. A verbal educational brief will also be provided to the subjects at this visit. The following screening laboratory analyses will be carried out:

- Serum HIV 1/2 antibody (between Days -14 to -2)
- Hepatitis B surface antigen (HBsAg) (between Days -14 to -2)
- Hepatitis C virus (anti-HCV antibody) (between Days -14 to -2)

If initial screening is within the -14-day window, it can count as both the initial screening and pre-vaccination visit.

A screening test for any of the above assessments may be repeated (total screening blood volume not to exceed 30 mL) in the event of a laboratory error (i.e., hemolyzed sample) or in the event the screening physician believes: 1) the laboratory test has identified a normal variant of a healthy state and 2) the variant is no more than a Grade 1 abnormality. This repeat screening applies to the initial screening tests [(executed between -90 to -2 days) and the repeat screening labs if executed (between days -14 to -2)].

The Walter Reed Army Institute of Research Clinical Trials Center (WRAIR CTC) may use a screening protocol in recruiting subjects for this study. All screening procedures outlined in this section may be completed under this screening protocol.

During this screening process, the laboratory assays will be reviewed to determine if any clinical laboratory abnormalities exist that would preclude study participation. Subjects who have only 1 mild (grade 1) abnormality may be included if the PI determines that their

participation will not present undue risk to the subject. Subjects with 2 or more mild abnormalities may be included in the study only with the consensus of the PI and the research monitor. Subjects with clinical laboratory abnormalities of greater than mild severity will not be eligible for this clinical trial.

The clinical toxicity grading scale that will be used as a guideline is based on guidance from the Center for Biologics Evaluation and Research (Guidance for Industry: Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials). Final grading determination will be made by the PI based on local normal lab value ranges for the specific lab and clinical symptoms. If any additional safety labs are performed, the FDA Guidance for Industry will be utilized.

7.4. Vaccination

After initial screening, eligible subjects will be vaccinated, dependent upon vaccine group assignment, with CssBA with or without dmLT via IM route to upper arms. The vaccine will be administered on days 1, 22, and 43 in accordance with formulation and vaccination procedures. IM injection of the vaccine will be given using a 1inch needle in the deltoid region (study clinician may choose another location on a case-by-case basis) by a study clinician.

7.4.1. Prior to Injection

Provisionally qualified subjects are scheduled to arrive at study site on the morning of Day 1 and will undergo the following procedures:

- Interval medical history is obtained, including medications.
- Vital signs are measured prior to dosing.
- A directed physical exam is performed.
- For female subjects, a urine pregnancy test performed.
- Blood samples are obtained for immunological testing.
- Pregnancy (if female of childbearing potential) test results from this visit and all other qualification-related laboratory test results from the screening visit(s) are reported and reviewed.
- New health information is reviewed to determine qualification for enrollment.
- Inclusion/Exclusion criteria are reviewed to assess continued eligibility.
- The prospective injection site is examined visually and by palpation.

7.4.2. Vaccine Administration

Approximately 50 qualified subjects will be enrolled for vaccination; if an insufficient number of qualified subjects is available to complete enrollment of the group, partial enrollment may occur, with the balance of enrollment to occur on later date(s). The injection site will be observed prior to dose administration (as above). If a qualified subject selected for enrollment is disqualified prior to enrollment/randomization and dose administration (e.g., withdraws consent or the PI reconsiders and disqualifies for a documented reason), another qualified subject will be selected for enrollment in place of the disqualified subject.

7.4.3. Post-Vaccination Observation Period

Subjects will remain at the site for at least 30 minutes post-injection. During this time, the subject will be provided with a Memory Aid, follow-up visit information, and contact telephone numbers for study staff; subjects will be instructed on the use of the materials.

After at least 30 minutes, the following procedures will be done:

- Vital signs will be measured.
- The injection site will be assessed and any findings will be recorded. Any complaints or reactogenicity signs or symptoms will be assessed and documented.
- The PI (or designee) may determine that a subject requires further on-site observation; additional site or clinical assessments may be completed as needed.

When all study-related procedures are complete and the PI (or designee) determines that a subject's condition is acceptable, the subject will be discharged from study site.

Subsequent immunizations will be performed on the alternate upper arm from the previous vaccination. An exception may be needed if there is pain/tenderness, erythema, edema, pruritus, induration, or rash at the planned site of vaccination. In this case, the investigator may exercise the discretion to administer the vaccination on the same arm as the previous vaccination. The site of vaccine administration will be recorded in the source documents.

7.5. Post-Vaccination Follow-up

Subjects will return for follow-up approximately 24 hours and 7 days after the first vaccination for a clinical evaluation which includes collection of vital signs, assessment of AEs, and review of changes in medical history, concomitant medications, and targeted clinical assessment. For subsequent vaccinations, each subject will undergo these same clinical evaluations at approximately 24 hours and 7 days after the second and third vaccinations. A symptom memory aid will be used by the subjects to monitor local vaccine site findings and symptoms and this will be reviewed at the follow-up visits.

Blood, saliva, and stool collections for safety and immunogenicity measures will be collected throughout the vaccination phase (See Study and Events Schedule; Table 8). For female subjects, a pregnancy test will be performed prior to each vaccination and on their last study visit.

Study clinicians will review the memory aid with each subject and use the memory aid to complete visit source documents (clinical encounter forms, AE forms) and progress notes. The clinician will review entries with subject and any changes made to entries will be initialed and dated by the subject.

Approximately 6 and 12 months from last vaccination, all subjects will be contacted via telephone to assess for a final safety assessment. In addition to soliciting history of any persistence of skin findings associated with vaccine sites, subjects will be asked about the diagnosis of any of the conditions included in the Medically Attended Adverse Events (MAAEs) Addendum. A history for any additional serious new diagnoses or hospitalizations will also be solicited. Identification of any of these conditions will be reported as an SAE per section 12.5.1.

Three attempts will be made and documented; after 3 unsuccessful attempts a certified letter and/or email will be used to request the subject to contact the study team.

7.6. Dose Escalation and Down-Selection

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 50), 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.

7.7. Study Endpoints

- **Safety**: Safety monitoring will be undertaken using in-person symptom surveillance, symptom memory aids, and targeted physical exams. AE monitoring will survey and specifically inquire about fever (oral temperature > 100.4^{o}F), malaise, headache, rash, pain, and extremity pain, swelling, or local reactions (further addressed in section 12.2.1.2). Clinical definitions will be used to grade the severity of symptoms in accordance to the following severity scale:

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

- **Immunology:** Seroconversion to LT and CS6 will be defined as a ≥ four-fold increase in endpoint titer between pre-and post-vaccination samples. Mucosal responses will be assessed similarly after adjusting for total IgA. A positive ALS response will require a four-fold rise in antibody titers between pre and post vaccination samples.

Table 8. Time and Events Schedule

Study Event	**Screen**	**-7**	**1**	**2**	**8**	**22**	**23**	**29**	**43**	**44**	**50**	**57**	**71**	**223**	**403**
Compliance Ranges	-90 to -2	-14 to -2	NA	NA	7-9 d	20-24 d	N/A	27-31 d	41-45 d	N/A	47-53 d	54-60 d	67-75 d	± 1 mo	± 1 mo
Study Briefing	X	X													
Comprehension Assessment	X														
Informed Consent	X														
Screening Medical History and Physical Examination	X														
CBC	X				X			X				X			
Chemistry[1]	X				X			X				X			
Anti-HIV-1/2		X													
HBsAg		X													
Anti-HCV		X													
Serum IgA	X														
Vital signs (BP, HR, T)	X	X	X	X	X	X	X	X	X	X	X	X	X		
Urine pregnancy test	X		X			X			X				X		
Vaccination			X			X			X						
Clinical check[2]	X	X	X	X	X	X	X	X	X	X	X	X	X		
Serology			X	X		X			X				X		
Saliva			X		X	X		X	X		X		X		
Stool			X		X	X		X	X		X		X		
Peripheral Blood Mononuclear Cells (PBMCs)[3]			X		X	X		X	X		X		X		
Study completion													X		
Post-study safety assessment (via telephone follow-up)														X	X
Approximate blood volume (ml) by study day[4]	**15**	**25**	**82**		**34**	**74**		**34**	**74**		**24**	**10**	**74**		
Approximate blood volume (ml) by study day[5]	**10**	**15**	**73**	**4**	**47**	**49**		**47**	**49**		**40**	**7**	**73**		

The data will be locked following entry of the day 71 data.

[1] Chemistry includes serum electrolytes, glucose, BUN, creatinine, AST, and ALT

[2] Clinical checks: baseline physical exam and medical history at screening, pre- and post-vaccination medical history and targeted physical exams (subjects will be observed for 30 minutes post-vaccination).

[3] PBMCs include collection for Antibody Secreting Cells (ASC), Antibody-Lymphocyte Supernatant (ALS), Memory-B cells. These samples may also be stored and utilized in future expanded immunology to include transcriptomics, proteomics, flow cytometry, and cytokine analysis.

[4] Total approximate blood volume to be collected for cohorts A and B: 446 ml

[5] Total approximate blood volume to be collected for cohorts C, D, and E: 414 ml

8. SELECTION AND WITHDRAWAL OF SUBJECTS

8.1. Recruitment

Healthy adult men and women, civilian and active-duty military, will be recruited from the Baltimore/Washington, DC, area through the WRAIR CTC by the use of advertisement in multiple media formats, to include but not limited to: newspapers, fliers, e-mails, the WRAIR CTC web site, public listservs, social media (such as Facebook), posters, bus ads, generic radio studies. E-mail announcements and web site postings will include information found on recruitment scripts (excluding any compensation information) or posters excluding any photos unless attached as a complete flyer. Recruitment may also include oral presentations at events, meetings, and briefings wherein the desired recruit population might reasonably be expected to attend. All forms of recruitment, printed media, handouts, and briefs will have IRB approval prior to being used.

When a subject calls the WRAIR CTC recruitment office and discloses an interest in the study, the recruitment staff will discuss details of the trial from a prepared, IRB-approved script. If the subject is still interested, contact information will be obtained and an appointment for briefing/screening will be arranged.

8.2. Subject Inclusion Criteria

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

- Healthy, adult, male or female, age 18 to 45 years (inclusive) at the time of enrollment.
- Completion and review of comprehension test (achieved $\geq$ 70% accuracy).
- Signed informed consent document.
- Available for the required follow-up period and scheduled clinic visits.
- 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.

8.3. Subject Exclusion Criteria

Subjects meeting any of the following criteria will be excluded from the study:

General Health

- 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.
- Clinically significant abnormalities on physical examination.
- 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).

- Women who are pregnant or planning to become pregnant during the study period plus 3 months beyond the last vaccination and currently nursing women.
- 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.
- Positive blood test for HBsAg, HCV, HIV-1/2.
- Clinically significant abnormalities on basic laboratory screening.

Research Specific

- Exclusionary skin history/findings that would confound assessment or prevent appropriate local monitoring of AEs, or possibly increase the risk of an AE.
- History of chronic skin disease (clinician judgment).
- Acute skin infection/eruptions on the upper arms including fungal infections, severe acne or active contact dermatitis.
- Allergies that may increase the risk of AEs.
- Regular use (weekly or more often) of antidiarrheal, anti-constipation, or antacid therapy.
- 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*

- History of microbiologically confirmed ETEC or cholera infection in the last 3 years.
- 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).
- 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.
- Vaccination for or ingestion of ETEC, cholera, or *E. coli* heat labile toxin within 3 years prior to dosing.
- Occupation involving handling of ETEC or *V. cholerae* currently, or in the past 3 years.

8.4. Subject Withdrawal Criteria

Each subject may withdraw consent at any time during the study without penalty. Counseling about the subject's health will be provided if he/she decides to discontinue participation in the study. Medical advice regarding what is in the best interest of the subject will be provided.

The PI may discontinue the subject's activity without the subject's consent if any of these criteria are met:

- A subject fails to comply with study procedures.
- A subject's safety or health may be compromised by further participation.
- It is determined to be in the subject's best interest.

8.4.1. When and How to Withdraw Subjects

A subject may end his or her participation in the study at any time. If a subject withdraws, the investigator will make a reasonable effort to determine the reason for the withdrawal from the study and to complete termination procedures as described in Section 8. Telephone calls, registered letters, and email correspondence are considered reasonable effort. For subjects leaving the study, a targeted examination may be performed, if medically indicated and if permitted by the subject.

A subject may be withdrawn for an adverse event (AE) or serious adverse event (SAE) resulting in a safety concern or for noncompliance with protocol requirements. When a subject withdraws due to an AE or is withdrawn by the PI due to an AE, the Sponsor must be notified within 72 hours.

Investigators must follow specific policy at each institution regarding the timely reporting of AEs and SAEs to the local IRB (section 12.5). In all cases, the PI will make a reasonable effort to complete study termination procedures.

If a subject meets withdrawal conditions for a concomitant medication violation or noncompliance, this should clearly be stated in the source document and the study termination eCRF.

8.4.2. Data Collected for Withdrawn Subjects

All data collected up to the time of withdrawal will be reported. The study termination CRF will be completed, with the reason for withdrawal specified.

8.4.3. Replacement of Subjects

Up to four alternates may be recruited for every group and asked to come to the day of first vaccination for each group. If an assigned subject does not present on the first day of vaccination, elects to withdraw, or is found to have met an exclusion criterion, an alternate will be enrolled.

Subjects who withdraw or are withdrawn after the first vaccination will not be replaced.

8.4.4. Follow-up for Withdrawn Subjects

Attempts will be made to follow all subjects for safety up to 28-days after receipt of the last vaccine dose. Short term safety follow up 7-days post vaccination will also be attempted. If unenrolled, attempts will be made to continue collecting specimens for immunological testing if subjects have received at least two doses of vaccine. Attempts will also be made to have the 6 and 12 month calls if the subjects are willing.

9. TREATMENT OF SUBJECTS

9.1. Description of Investigational Products

Table 9. Investigational Products

Product Name	**CssBA (Lot 1880)**	**dmLT (Lot 001 08 16)**
Dosage Form	5, 15, or 45 μg on days 1, 22, 43	100 or 500 ng on days 1, 22, and 43
Route of Administration	IM	IM
Physical Description	Clear to slightly hazy, homogenous liquid	White to off-white uniform dense cake
Manufacturer	WRAIR PBF, Bldg 501, 501 Robert Grant Avenue, Silver Spring, Maryland 20910	IDT Biologika Corporation 1405 Research Boulevard Rockville, MD 20850

9.2. Concomitant Medications

Subjects taking regular medication (i.e., birth control pills or multivitamins) prior to enrollment in the trial will be allowed to continue to take this medication unless it is specifically excluded as part of the inclusion/exclusion criteria for the trial. Subjects needing to take non-approved or excluded medication will not be eligible for enrollment in this study. Any medication ordered by the study physician during the course of the trial, such as a topical steroidal cream, will be documented on the concomitant medication page of the source documents. Approved medications that were being taken prior to, as well as during the course of the trial will also be documented in this manner.

9.3. Treatment Compliance

All vaccines will be administered by a study investigator or research nurse.

10. INVESTIGATIONAL PRODUCTS AND MANAGEMENT

10.1. Investigational Products

The investigational products are CssBA (Lot 1880, manufactured at the WRAIR PBF) and dmLT, manufactured at IDT Biologika Corporation (comparable to lot being tested in the toxicology study and final lot # release Aug 2016). Products have passed tests for sterility, purity, endotoxin content, appearance, pH, and protein concentration. In addition, all three have passed General Safety Tests in mice. Table 9 presents a summary description of the investigational products.

10.2. Investigational Product Packaging and Labeling

The investigational products will be covered under an Investigational New Drug (IND) application. Each vial is labeled for human administration and includes the following statement: "New Drug – Limited by Federal Law to Investigational Use." IND product components are listed below:

- CssBA (Lot 1880) vial label.

Recombinant
spd_dsc$_{16B}$ntd$_{14}$CssBA$_{B7A}$[His]$_6$ Protein
BPR No.: BPR-1152-00 **Lot No.: 1880**
Contents: 0.70 ml Storage: -80 ± 10 °C
Caution: New drug - Limited by Federal (or United States) law to investigational use.
Date of Mfg.: 20 Jun 14
Mfg. By: WRAIR, Silver Spring, MD 20910

- dmLT (comparable to lot being tested in the toxicology study and final lot # release Aug 2016): vials.

  - Reconstructed label from vial of dmLT:

Recombinant double mutant Heat Labile Toxin (dmLT) LT (R192G/L211A) Expressed in E.coli Contents: 0.5 ml (1 mg/ml, Lyoph.) Storage ≤-10˚C Caution: New drug limited by Federal Law To investigational use only Manufactured by: IDT, Rockville, MD 20850 IDT Biologika GmbH, Am Pharmpark, 06861, Dessau Roßlau	Lot #: 001 08 16 MFG Date: 08/2016

10.3. Investigational Product Storage

CssBA vials are stored at -80º±10ºC and dmLT vials are stored at <-10°C.

The day prior to vaccination, the required number of CssBA vials will be moved to overnight storage at the CTC. The CssBA will be stored at 2-8^{o}C to allow the frozen liquid to thaw. If it is not possible to move CssBA vials to 2-8^{o}C for overnight storage prior to vaccination day, it is acceptable to rapid thaw the vials at room temperature. The dmLT will be maintained <-10°C until ready for use.

10.4. Investigational Product Preparation

On the day of vaccination, the products will be used to formulate the appropriate vaccine preparations for the clinical groups. The vaccine will be used within 6 hours of preparation. Vaccine or open vials will not be saved for use on another study day. Any remaining vaccine product will be properly disposed of in accordance with Manual of Procedures (MOP). Vaccine formulation for each of the groups is described in detail in the MOP.

10.5. Investigational Product Accountability

Personnel in the Enteric Disease Department at the Naval Medical Research Center and the clinical coordinator staff at the CTC will be responsible for product accountability. The PI is responsible for and will maintain logs of investigational product receipt, storage, reconstitution, accountability by subject, and investigational product remaining before final disposition. At the CTC, the logs will be maintained in the accountability files. The PI may delegate, in writing, this responsibility to another individual, but the PI is ultimately responsible for the investigational product and its proper storage upon receipt at the study site.

All unused or partially used investigational product and empty vials will be destroyed or used for non-clinical research purposes as per the MOP.

11. LABORATORY AND CLINICAL ASSESSMENTS

Laboratory samples described below will be collected at times specified in Table 8.

11.1. Sample Collection

11.1.1. Blood Sample Collection

Study serology and immunological analyses will be performed from blood. Peripheral Blood Mononuclear Cells (PBMCs) will also be collected to determine Antibody responses from Lymphocyte Supernatant. Blood samples for immunology will be separated into plasma and lymphocyte fractions using a FICOLL- HYPAQUE gradient technique with plasma stored at -70°C (±10°C) and lymphocytes not used on the day of collection stored in vapor phase liquid nitrogen at NMRC until testing is initiated.

11.1.2. Stool Sample Collection

Stool will be collected from subjects for immunogenicity testing for fecal IgA. Subjects will be provided stool hats to self-collect all stools which will be processed within two hours of receipt in the CTC and within 8 hours of collection by the subject, according to specific study procedures that have been utilized successfully for other enteric disease studies.

11.1.3. Saliva Sample Collection

Collection of saliva samples will be performed utilizing synthetic oral swabs (Salimetrics Oral Swab; SOS). The subject will place a single swab in their mouth under the tongue to collect saliva for 10 minutes. Subjects will be instructed not to eat or drink anything, including chewing gum, for 10 minutes prior to saliva sample collection. Subjects will be instructed to avoid drinking alcohol or using mouthwash for 24 hours and to avoid caffeinated beverages for 12 hours prior to collecting the sample. Saliva collection vials will be pre-loaded with 10uL of 100X HALT Protease Inhibitor Cocktail.

11.2. Specification of Safety Endpoints

11.2.1. Demographic/Medical History

During initial screening, a medical history is obtained before Study Day 1. Approximately 7 days prior to vaccination (allowable range: day -14 to -2), subjects will have a follow-up medical history. During subsequent vaccination and follow-up visits, a review for changes in medical history will be performed.

11.2.2. Vital Signs

Vital signs (oral temperature, blood pressure, and heart rate) will be obtained at each clinic visit.

Table 10. Reference Ranges and Adverse Event Coding for Vital Signs Parameters

Vital Signs	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)
Heart rate				
Tachycardia	101–115	116-130	>130	ER visit or hospitalization for arrhythmia
Bradycardia	50-54[a]	45–49	<45	ER visit or hospitalization for arrhythmia
Fever (°C) (°F)	≥100.4 °F and ≤101.1°F (38.0-38.4°C)	≥101.1°F and ≤102.0°F (38.5-38.9°C)	≥102.1 °F and ≤104 °F (39.0-40.0 °C)	> 104.0 °F; life threatening
Blood Pressure				
Hypertension (systolic, mm Hg)	141–150	151 - 155	>155	ER visit/hospitalization for malignant hypertension
Hypertension (diastolic, mm Hg)	91–95	96 – 100	>100	ER visit/hospitalization for malignant hypertension
Hypotension (systolic, mm Hg)[b]	85–89	80 – 84	<80	ER visit/hospitalization for hypotensive shock

[a] Grade 1 bradycardia will not be considered an abnormality for this study unless judged to be clinically significant by the PI or the PI in consultation with the Research Monitor and sponsor.

[b] If a subject has a baseline systolic BP in the 90's then a decrease in BP < 10 without associated clinical symptoms will not be considered an abnormality for this study unless judges to be clinically significant by the PI.

11.2.3. Physical Examination

A complete physical exam will be conducted during the screening visit as part of the screening process. Subsequent focused clinical examinations will occur at each study visit with specific attention to the identification of local, systemic or other adverse reactions.

11.2.4. Laboratory Assessments

Venous blood samples will be collected for chemistry, hematology and immunological parameters. Hematology and Chemistry analyses (BUN, creatinine, AST, ALT, electrolytes, glucose) will occur at time times indicated in Table 8. Hematology and chemistry analyses will be performed by commercial laboratory (Quest, Incorporated). Additional specimens may be collected to confirm and evaluate any abnormal values.

The clinical toxicity grading scale that will be used as a guideline is based on the Guidance for Industry: Toxicity Grading Scale for Healthy Adult and Adolescent Subjects enrolled in Preventive Vaccine Clinical Trials. Final grading determination will be made by the PI based on normal lab values for the specific lab and clinical symptoms. If any additional safety labs are performed, we will utilize the FDA Guidance for Industry.

11.2.4.1. Hematology

The following hematology parameters will be assessed:

- White blood cell count
- Red blood cell count
- Platelet count

- Hemoglobin

Table 11. Reference Ranges and Adverse Event Coding for Clinical Hematology Parameters

Test	Quest Normal	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)
Hemoglobin (g/dL) (for screening purposes only)	M: LLN = 13.2 F: LLN = 11.7	M: 12.5-13.1 F: 11.0-11.6	M: 10.5-12.4 F: 9.5-10.9	M: 8.5-10.4 F: 8.0-9.4	M: <8.5 F: <8.0
Hemoglobin - decrease from lower limit of normal (used to grade toxicity)		0.5-1.5	1.6-2.0	2.1-5.0	> 5.0
Neutrophils (cells/mm^3)	1,500-7,800	1,225-1,499	1,000-1,224	776-999	< 776
Leukocytes (white blood cells) (cells/mm^3)	3,800-10,800				
Leukopenia		2,500-3,799	1,500-2,499	1,000-1,499	< 1,000
Leukocytosis		10,801-15,000	15,001-20,000	20,001-25,000	> 25,000
Lymphocytes (cells/mm^3)	850-3,900	750-849	500-749	250-499	< 250
Eosinophils (cells/mm^3)	15-500	551-1,500	1,501-5,000	> 5,000	Hypereosinophilic
Platelets decreased – $10^3/mm^3$	140-400	125-139	100-124	25-99	< 25

11.2.4.2. Blood Chemistry

The following clinical chemistry parameters will be assessed:

- Serum electrolytes
- Glucose
- BUN
- Creatinine
- AST
- ALT

Table 12. Reference Ranges and Adverse Event Coding for Blood Chemistry Parameters

Test	Quest Normal	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)
Sodium	135-146 (mmol/L)				
Hyponatremia		132-134	130-131	125-129	< 125
Hypernatremia		147-148	149-150	151-152	> 152

Potassium	3.5-5.5 (mmol/L)				
Hypokalemia		3.3-3.4	3.1-3.2	2.9-3.0	< 2.9
Hyperkalemia		5.6-5.7	5.8-5.9	6.0-6.1	> 6.2
Glucose, Random	65-139 (mg/dL)				
Hyperglycemia		140-155	156-200	> 200	Insulin requirements or hyperosmolar coma
Hypoglycemia		60-64	55-59	45-54	< 45
SGOT/AST (elevation)	M: 10-40 U/L F: 10-30 U/L	M: 41-100 F: 31-75	M: 101-200 F: 76-150	M: 201-400 F: 151-300	M: > 400 F: > 300
SGPT/ALT (elevation)	M: 9-60 U/L F: 6-40 U/L	M: 61-150 F: 41-100	M: 151-300 F: 101-200	M: 301-600 F: 201-400	M: > 600 F: > 400
BUN (elevation)	7-25	26-28	29-31	> 31	Requires dialysis
Creatinine (elevation)	M: 0.7-1.4 F: 0.5-1.1	M: 1.5-1.7 F: 1.2-1.7	M: 1.8-2.0 F: 1.8-2.0	M: 2.1-2.5 F: 2.1-2.5	M: >2.5 F: >2.5 or requires dialysis

11.2.4.3. Virus Serology

Serologic evidence of chronic HIV-1/2, HCV and HBV infections will be obtained during the screening process no earlier than 14 days before initial vaccination. Evidence of infection would make a subject ineligible. Additional testing will not be performed as part of this study.

11.2.4.4. Pregnancy Screen

A urine sample for pregnancy testing will be collected at the screening visit for female subjects; Pregnancy tests will also be obtained on each day of vaccination, and at the final study visit. A positive pregnancy test prior to vaccination will result in no additional study vaccinations being provided.

Any subjects who become pregnant during the study will have follow-up safety labs (as appropriate) prior to being removed from the study. The subject will then be removed from the study and followed until the end of their pregnancy. Procedures to be followed in the event a study participant becomes pregnant during the study period are outlined below in Section 12.5.2.1.

11.2.5. Local Reactions

Symptoms and signs will be specifically surveyed for at each visit as well as by patient self-report using a symptom memory aid tool. Specifically, the following symptoms will be solicited: local pain, pruritus, induration, erythema and other rash/skin lesions as well as any systemic signs and symptoms. Local reactions will be coded for severity based on the severity scale outlined in Section 12.4.3.

Photograph and Measuring Tool: A baseline photograph of the vaccination site may be obtained prior to vaccination, one day post-vaccination and, in the event of a skin rash, every 7 days until resolution or study completion. The subject may be photographed in order to record and follow any vaccine site or other local reactions. These photographs will not be part of statistical analysis of study endpoints but rather may serve for internal monitoring and follow up of any reactions. Photograph files will be maintained at the Enterics Department.

11.3. Immunology

11.3.1. Primary Immunology

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. Reciprocal endpoint titers < 5 will be assigned a value of 2.5 for computational purposes. Seroconversion will be defined as a ≥ 4-fold increase in endpoint titer between pre-and post-vaccination samples AND a post-vaccination reciprocal titer >10.

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. A positive ALS response will require a four-fold rise in antibody titers between pre and post vaccination samples. For each antigen, pre-and post-challenge samples will be tested concurrently.

11.3.2. Exploratory Immunology

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.

Samples will be collected as outlined in the time and events schedule. In order to support additional future exploratory evaluations in systems biology, cells and serum samples will be collected for use in a variety of transcriptomic, proteomic, flow cytometry, memory B cells, and cytokine analysis. Any testing of samples for these exploratory outcomes will be performed under a modification to this protocol to further detail the assay(s) or performed under a separate research protocol.

12. ASSESSMENT OF SAFETY

Safety monitoring will be conducted throughout the study; therefore, safety concerns will be identified by continuous review of the data by the PI, clinic staff, clinical monitor, Research Monitor, and SRC.

The SRC will be comprised of one Independent Safety Monitor (ISM) and a committee of outside members with expertise in early phase 1 clinical trials. The SRC will be established under a charter and function to offer their expertise, complete expedited review of safety reports, review safety data prior to dose escalation, make recommendations about as to the study continuation, and advise the study sponsor regarding trial safety.

Study Safety Management: The IRBs, Research Monitor, SRC, and PI will review any safety concerns.

Research Monitor: The Research Monitor will function as an independent safety advocate for subjects per AR 70-25, DoD Instruction 3216.2. An independent Research Monitor is required to review all unanticipated problems involving risk to subjects or others, SAEs, and all subject deaths associated with the protocol and provide an unbiased written report of the event. At a minimum, the Research Monitor should comment on the outcomes of the event or problem and, in the case of a SAE or death, comment on the relationship to participation in the study. The Research Monitor should also indicate whether he/she concurs with the details of the report provided by the study investigator. Reports for events determined by either the investigator or Research Monitor to be possibly or definitely related to participation and reports of events resulting in death should be promptly forwarded to the IRB and the Sponsor.

12.1. Safety Parameters

12.1.1. Safety Criteria for Dose Escalation or Stopping Doses

The study will use a dose -escalation design to evaluate increasing doses of CssBA in combination with increasing doses of dmLT.

The PI, along with the Research Monitor and SRC, may determine a subject's local site reactions warrant discontinuation of receiving subsequent vaccinations. If any of the following events occurs, administration of investigational product will be discontinued until a thorough review of the events is undertaken by the investigators and the SRC:

- The occurrence of one or more serious adverse events determined to be related to the investigational vaccine (definitely, probably or possibly) within the 72 hour period post-vaccination.
- Severe diarrhea, defined as six or more liquid stools within 24 hours of vaccination.
- A severe local rash, defined as one that makes the subject unable to perform normal daily activities and which is not attributable to another cause.
- Systemic rash, including but not limited to generalized urticaria, generalized petechiae, or erythema multiforme, occurring in two or more subjects in a group will result in stopping further vaccination in subjects groups.

- One vaccine-related serious or unexpected AE evaluated by the PI, Research Monitor, and sponsor's representative and determined to be an unacceptable risk to the health and safety of other investigational product recipients.

Further vaccination, in accordance with the protocol, may be resumed with the concurrence of the SRC, sponsor, PI, and the US Food and Drug Administration (FDA).

If it is determined that a subject will not continue with future vaccine doses, they will remain enrolled in the study for follow-up.

An interim safety analysis for Group A will be prepared and reviewed to include all safety data through 7 days after the third vaccination dose for Group A. This report will be provided to the SRC after Day 50 for their review to determine whether the study can continue to enroll for Group B or whether the study should be stopped. An interim safety analysis for Group B, C, and D will be prepared and reviewed to include all safety data through 7 days after the third vaccination dose. This report will be provided to the SRC after Day 50 for their review to determine whether the study can continue to enroll for the next, dose-escalating group or whether the study should be stopped. In addition to the interim safety analyses, the PI, along with the SRC will stop the study until further review by the SRC if any of the following criteria that are thought to be related to the investigational vaccine are identified:

- One or more grade 3 laboratory abnormalities.
- Two or more grade 2 laboratory abnormalities.
- Any serious adverse event (SAE) occurring at any time during the study duration and assessed as at least possibly related to study vaccination.
- Any non-serious unexpected AE related to study vaccination and determined to be an unacceptable risk to the health and safety of other investigational product recipients.
- The same or similar severe (grade 3 or higher) solicited or unsolicited AEs assessed as at least possibly related to study vaccination reported by 10% or more subjects in the 7 days following vaccination.

If the study were to be stopped, subjects would be followed for the safety assessments but no additional vaccinations would be given (unless otherwise specified by the SRC).

12.1.2. Criteria for Study Termination

The PI, the Research Monitor, the SRC, IRB, sponsor, or the FDA may stop or suspend the use of the products at any time.

12.2. Adverse and Serious Adverse Events

12.2.1. Definition of Adverse Events

12.2.1.1. Adverse Event (AE)

An AE, as defined by the ICH guideline for GCP, is:
"**Adverse event** means any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related.

Suspected adverse reaction means any adverse event for which there is a reasonable possibility that the drug caused the adverse event. For the purposes of IND safety reporting," reasonable possibility means there is evidence to suggest a causal relationship between the drug and the adverse event. Suspected adverse reaction implies a lesser degree of certainty about causality than adverse reaction, which means any adverse event caused by a drug."

An AE is considered to be any adverse change or exacerbation from a baseline condition which occurs following the initial administration of an investigational product whether or not the event is considered to be related to the investigational product. Examples of this include but are not limited to the following:

- Adverse changes including new signs and symptoms, intercurrent illness modifying the clinical course, or the worsening of a baseline condition including the increased frequency of an event or an increased intensity of a condition.
- Concomitant disease with onset or increased severity after the start of study product administration.
- A new pattern in a pre-existing condition, occurring after the receipt of investigational product that may signal a clinically meaningful change.
- Clinically significant changes in laboratory values.

Medically attended adverse events (MAAEs) are adverse events that include potential immune-mediated medical conditions as listed in the MAAEs Addendum.

12.2.1.2. Solicited Adverse Events

A solicited AE is a predetermined event, identified in the Investigator's Brochure, which may reflect safety concerns related to the investigational product. The solicited AEs for this study include:

1. Vaccine Site Pain
2. Vaccine Site Pruritus
3. Vaccine Site Rash/eruption
4. Vaccine Site Swelling (As reported by subject)
5. Vaccine Site Tenderness
6. Fever (subjective or objective)
7. Headache
8. Loose Stools
9. Arthralgia
10. Myalgia
11. Malaise
12. Nausea
13. Vomiting

Cutaneous evidence of systemic allergic reactions such as urticaria or angioedema will be also documented. Subjects will also be evaluated for development of regional lymphadenopathy. A photograph may be taken of local vaccine site reactions for internal use. Systemic signs and symptoms will also be documented in detail and evaluated as clinically appropriate.

Abnormalities will be described in detail in order to monitor progress. This will include the nature of the adverse event, the date and time of onset and offset (duration), severity, and PI determination of relationship to the investigational vaccine.

12.2.1.3. Serious Adverse Event (SAE) or Serious Suspected Adverse Reaction

An adverse event or suspected adverse reaction is considered "serious" if, in the view of either the investigator or sponsor, it results in any of the following outcomes:

- Death.
- Life-threatening adverse event.
- Inpatient hospitalization or prolongation of existing hospitalization.
- Persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions.
- Congenital anomaly/birth defect.

An adverse event or suspected adverse reaction is considered "life-threatening" if, in the view of either the investigator or sponsor, its occurrence places the patient or subject at immediate risk of death. It does not include an adverse event or suspected adverse reaction that, had it occurred in a more severe form, might have caused death.

Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. 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.

12.2.1.4. Unexpected Adverse Event

An adverse event or suspected adverse reaction is considered "unexpected" if it is not listed in the investigator brochure or is not listed at the specificity or severity that has been observed; or, if an investigator brochure is not required or available, is not consistent with the risk information described in the general investigational plan or elsewhere in the current application, as amended. For example, under this definition, hepatic necrosis would be unexpected (by virtue of greater severity) if the investigator brochure referred only to elevated hepatic enzymes or hepatitis. Similarly, cerebral thromboembolism and cerebral vasculitis would be unexpected (by virtue of greater specificity) if the investigator brochure listed only cerebral vascular accidents. "Unexpected," as used in this definition, also refers to adverse events or suspected adverse reactions that are mentioned in the investigator brochure as occurring with a class of drugs or as anticipated from the pharmacological properties of the drug, but are not specifically mentioned as occurring with the particular drug under investigation.

12.2.1.5. Serious and Unexpected Suspected Adverse Reactions (SUSARs)

Adverse events which are Serious and Unexpected, and which are felt to be at least possibly related to the investigational agent(s) or procedures may be of particular importance for reporting.

12.2.1.6. Other Adverse Event (OAE)

Other adverse events will be identified by the PI during the evaluation of safety data. Significant adverse events of particular clinical importance, other than SAEs and those AEs leading to discontinuation of the subject from the study, will be classified as other adverse events. For each, a narrative may be written and included in the clinical study report.

12.3. Relationship to Investigational Products

The PI must assign a relationship of each AE to the receipt of the investigational product. The investigator will use clinical judgment in conjunction with the assessment of a plausible biologic mechanism, a temporal relationship between the onset of the event in relation to receipt of the investigational product, and identification of possible alternate etiologies including underlying disease, concurrent illness or concomitant medications. The following guidelines will be used by investigators to assess the relationship of an AE to study product administration.

- Not related: No relationship to investigational product. Applies to those events for which evidence exists that there is an alternate etiology.
- Unlikely related: Likely unrelated to the investigational product. Likely to be related to factors other than investigational product, but cannot be ruled out with certainty.
- Possibly related: An association between the event and the administration of investigational product cannot be ruled out. There is a reasonable temporal association, but there may also be an alternative etiology such as the subject's clinical status or underlying factors including other therapy.
- Probably related: There is a high degree of certainty that a relationship to the investigational product exists. There is a reasonable temporal association, and the event cannot be explained by known characteristics of the subject's clinical state or factors including other therapy.
- Definitely related: An association exists between the receipt of investigational product and the event. An association to other factors has been ruled out.

12.4. Recording Adverse Events

12.4.1. Methods/Timing for Assessing, Recording, and Analyzing Safety Endpoints

AEs, solicited AEs, and SAEs will be assessed at all study visits, documented in the source records, and recorded on the eCRFs using accepted medical terms and/or the diagnoses that accurately characterize the event. When a diagnosis is known, the AE term recorded on the eCRF will be the diagnosis rather than a constellation of symptoms. The investigator will assess all AEs for seriousness, relationship to investigational product, severity, and other possible etiologies. When an event has not resolved by the prescribed reporting period, it will be documented on the AE eCRF as “unknown”.

The timeframe for the collection of AEs and SAEs begins at the first administration of investigational product through 28 days after the last dose of investigational product is administered. Additionally, subjects will be contacted by telephone approximately 6 and 12 months after their final study vaccination to assess for any autoimmunity associated SUSARs and new onset SAEs. During the long-term follow-up phone call, subjects will be asked if they have been diagnosed with any of the medical conditions listed in the MAAEs Addendum.

12.4.2. Duration of Follow-Up of Subjects after Adverse Events

Investigators are required to follow AEs and SAEs to resolution, even if this extends beyond the prescribed reporting period. Resolution is the return to baseline status or stabilization of the condition with the probability that it will become chronic. The SAE outcomes will be reported to the sponsor's representative (see below) using the Supplemental SAE Report Forms.

PATH MEDICAL OFFICER:	Rahsan Erdem, MD Senior Medical Officer, Center for Vaccine Innovation & Access 455 Massachusetts Avenue NW, Suite 1000 Washington, DC 20001 Email: rerdem@path.org, T: 202.540.4546

Investigators are not obligated to actively seek SAEs in former subjects; however, if a SAE, considered to be related to the investigational products is brought to the attention of the investigator at any time following completion of the study, the event will be reported to the sponsor's representative as defined in section 12.5.1.1.

12.4.3. Severity Assessment

All AEs will be assessed for severity by the investigator. Inherent in this assessment is the medical and clinical consideration of all information surrounding the event including any medical intervention required. Each event will be assigned one of the following categories: mild, moderate, severe, or life-threatening. See section 11.2.2 for vital signs and section 11.2.4 for Laboratory values for further guidance in the assignment of severity. The criteria below may be used for any symptom not included in the grading scale. Any grade 4 (life-threatening) AE must be reported as an SAE. The eCRF for AEs will reflect only the highest severity for continuous days an 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 (i.e., 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 pose a threat to a subject's life or functioning. Seriousness (not severity) serves as a guide for defining regulatory reporting obligations.

12.5. Reporting Adverse Events

The PI will report all AEs to the sponsor's representative and the local IRB in the appropriate safety, annual, and/or final reports. The investigators will prepare annual and final reports to the FDA in consultation with the sponsor's representative.

12.5.1. Reporting Serious and Unexpected Adverse Events

Contact information for reporting SAEs is provided in Table 13.

12.5.1.1. Reporting to the Sponsor

Table 13. Study Contacts for Reporting Serious Adverse Events

Sponsor's Representative	The EMMES Corporation 401 N. Washington St., Suite 700 Rockville, MD 20850
Sponsor's Medical Officer	Rahsan Erdem, MD 455 Massachusetts Ave NW Suite 1000 Washington, DC 20001 Telephone: 202-540-4546 Fax: 202-457-1466 Email: rerdem@path.org
Institutional Review Board	Naval Medical Research Center Research Services Directorate, Office of Research Administration Code 025, Building 500, Rm 004 Silver Spring, MD Telephone: 301-319-7276 Fax: 301-319-7277
Research Monitor	Michael Koren, MD Walter Reed Army Institute of Research 503 Robert Grant Avenue Silver Spring, MD 20910 Telephone: 301-319-9904 michael.a.koren2.mil@mail.mil

Table 14. SAE Information to be Reported to the Sponsor's Representative

Notification Method	Information to be Provided
Email or Telephone (within 72 hours)	IND number, sponsor study number, name of the investigational product, and investigator name and contact number Subject identification number SAE, onset date, event term, date of investigational product administration, severity, relationship, and subject's current status
AND **Email or Fax**	Cover sheet or letter Adverse event case report form Supplemental SAE report forms Concomitant medication case report form or a list of concomitant medications

Notification Method	Information to be Provided
	Medical record progress notes including pertinent laboratory/diagnostic test results
NOTE: When submitting SAE reports via email, the subject line of each email notification will read as follows: **SAFETY REPORT – IND # _____, Sponsor Study #_____, Subject# _____, Event term: _____**	

In order to comply with regulations mandating sponsor notification of specified SAEs to the FDA within 7 calendar days, investigators must submit additional information as soon as it is available. The sponsor's representative will report unexpected SAEs associated with the use of the drug to the FDA as specified at 21 CFR 312.32 (c).

Investigators must follow all relevant regulatory requirements as well as specific policy at each institution regarding the timely reporting of SAEs to the local IRB, Research Monitor, and other applicable regulatory agencies.

Reporting to the sponsor's representative does not fulfill the investigator's duty to report all unanticipated problems involving risk to human subjects or others to the IRB. The PI will notify the local IRB, the Research Monitor, and other applicable groups, if necessary.

12.5.1.2. Reporting to the IRB

It is NMRC's policy to report to the IRB within 24 hours after determination of the event. A formal written report is due within 5 days of AE reporting.

Following AEs should be considered as unanticipated problems that must be reported to the NMRC IRB:

- Single occurrence of a serious, unexpected, and uncommon event that is strongly associated with investigational products exposure.
- A single or small number of a serious, unexpected event that is not commonly associated with investigational products exposure but is uncommon in the study population.
- Multiple occurrences of an AE that, based on aggregate analysis, is determined to be an unanticipated problem. There should be a determination that the series of AEs represents a signal that the AEs were not just isolated occurrences and involve risk to human subjects.
- An AE that is described in the investigator's brochure, protocol, or informed consent documents, but occurs at a specificity or severity that is inconsistent with prior observations.
- A serious AE that is described in the investigator's brochure, protocol, or informed consent documents, but for which there is a clinically significant increase in the expected rate of occurrence.

- Any other AE or safety finding, including those based on animal or epidemiologic data that would cause the sponsor to modify the investigator's brochure, protocol, or informed consent documents, or would prompt other action by the IRB to ensure the protection of human subjects.

All SAEs will be reported to the Western Institutional Review Board (WRIB) by the sponsor according to the WIRB guidelines and using the WIRB Promptly Reportable Information Form.

WIRB Phone: 800-562-4789, Fax: 360-252-2498.

PVS is required to report SAEs that fit the following criteria within 5 working days of the time of becoming aware of them:

- New or increased risk
- Protocol deviation that harmed a subject or placed subject at risk of harm
- Protocol deviation made without prior IRB approval to eliminate an immediate hazard to a subject
- Audit, inspection, or inquiry by a federal agency
- Written reports of federal agencies (e.g., FDA Form 483)
- Allegation of Noncompliance or Finding of Noncompliance
- Breach of confidentiality
- Unresolved subject complaint
- Suspension or premature termination by the sponsor, investigator, or institution
- Incarceration of a subject in a research study not approved to involve prisoners
- Adverse events or IND safety reports that require a change to the protocol or consent
- State medical board actions
- Unanticipated adverse device effect
- Information where the sponsor requires prompt reporting to the IRB

12.5.2. Reporting Additional Immediately Reportable Events to the Sponsor's Representative

12.5.2.1. Pregnancy

Each pregnancy must be reported *immediately* **(within 72 hours of identification)** by email or fax to the sponsor's representative and the NMRC IRB.

Subjects who become pregnant within 3 months after their last dose of investigational product will be followed to the end of their pregnancy, and information will be gathered for outcome, date of delivery, health status of the mother and child including the child's gender, height and weight. Complications and/or abnormalities should be reported, including any premature terminations. A pregnancy is reported as an AE or SAE only when there is suspicion that the investigational product may have interfered with the effectiveness of contraception or there was a serious complication in the pregnancy including a spontaneous abortion or an elective termination for medical rationale.

12.5.2.2. AE-Related Withdrawal of Consent

Any AE-related withdrawal of consent during the study must be reported *immediately* **(within 72 hours of identification)** by email or fax to the sponsor's representative and NMRC IRB.

12.5.2.3. Pending Inspections/Issuance of Reports

The knowledge of any pending compliance inspection/visit by the FDA, Office for Human Research Protections (Department of Health and Human Services), or other government agency concerning clinical investigation or research, the issuance of Inspection Reports, FDA Form 483, warning letters, or actions taken by any regulatory agency including legal or medical actions and any instances of serious or continuing noncompliance with the regulations or requirements will be reported immediately to the sponsor's representative.

13. KNOWN AND POTENTIAL RISKS AND BENEFITS TO HUMAN SUBJECTS

13.1.1. Risks/Discomforts to Subjects and Precautions to Minimize Risk

Outlined as follows are anticipated and unexpected adverse reactions and a brief description of procedures to ameliorate risks and symptoms. All known risks and precautions described here are explained in detail in the informed consent. In general, dose escalation is being performed to ensure an acceptable safety record for IM administration of vaccine products in this study. Prior to proceeding with the next dose in the next cohort of subjects, all safety data will be reviewed by the SRC, and a determination will be made as to whether the study can continue to enroll (at the dose escalation planned) based on the observed safety record from the preceding dose level. In addition to this overall safeguard, individual risks will be closely monitored and any AEs will be quickly identified and managed.

13.1.1.1. Local Reactions

Local reactions at the vaccination site are expected from the IM immunization. IM vaccination, in general, has been shown to be a safe and effective route of administration. There may be some mild discomfort and irritation from the initial injection. These are typically mild and transient in nature. In addition, there is the possibility of local erythema, pruritus, swelling, and/or induration that appears over time. In addition, subjects may develop temporary swelling in the lymph nodes under the arm where vaccination occurred.

13.1.1.2. Systemic Reactions

Any vaccine may be associated with a wide range of systemic reactions, such as fevers, constitutional symptoms (fatigue, malaise, appetite change), and gastrointestinal symptoms (diarrhea, abdominal pain) as well as others. The frequency and type of systemic symptoms will be assessed and analyzed with respect to study products.

13.1.1.3. Pregnancy

During initial screening and prior to each vaccination, a urine pregnancy test will be performed. Subjects with a positive test will be excluded from the study. A final urine pregnancy test will be conducted during the last clinic visit. While female subjects will not be required to disclose their method of birth control, they will be counseled during the initial screening on acceptable forms of birth control required for study enrollment. At each visit, female subjects will be reminded that they must agree to use effective contraception to remain in the study. Female subjects should not become pregnant during the study and for at least 3 months after the last vaccine dose.

13.1.1.4. Lactation

Risks to nursing infants are unknown at this time; breastfeeding females will be excluded from this study. Lactating females must agree not to breastfeed during the study and for 3 months after the last dose of study vaccination/dose of investigational product.

13.1.1.5. Venipuncture

Blood sampling carries a minimal risk of minor discomfort and the possibility of minor bruising at the site of the needle puncture and, rarely, the possibility of infection at the needle puncture site.

13.1.1.6. Allergic Reaction

As with any Investigational New Drug (IND) product administration and no matter what precautions are taken, there is always the risk of a serious, or even life-threatening, allergic reaction. Although anaphylactic reactions are not anticipated, as with any vaccination, the rare possibility exists. For this reason, all subjects will be observed at the study site WRAIR CTC or a minimum of 30 minutes following vaccine dosing. Appropriate emergency equipment (e.g., automated external defibrillator, air-shields manual breathing unit bag) and medication [e.g., antihistamines and adrenaline (epinephrine)] for initial treatment of an allergic reaction will be available at the WRAIR CTC whenever immunizations are given. This equipment is available to handle emergencies such as anaphylaxis, angioedema, bronchospasm, and laryngospasm.

14. STATISTICS

Detailed statistical procedures, listings, table shells and figures will be provided in a separate statistical analysis plan (SAP). The following key statistical components will be considered and a detailed description will be documented in the SAP:

- Primary and secondary endpoints and how they will be measured,
- Statistical methods and tests that will be used to analyze the endpoints,
- Strategy that will be used if the statistical test assumptions are not satisfied,
- Indication of whether the comparisons will be one-tailed or two-tailed (with justification of the choice) and the level of significance to be used,
- Identification of whether any adjustments to the significance level or the overall p value will be made to account for any planned or unplanned subgroup analyses or multiple testing,
- Specification of potential adjusted analyses and a statement with which covariates or factors will be included,
- Planned exploratory analyses and justification of their importance, and
- Any subgroup effects with biological justification and support from within and outside the study.

14.1. Data Analysis

The primary study objective is safety. 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.

Rates of all MAAEs identified during the long term telephone follow-up calls 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. MAAEs data will be listed individually and summarized by type of disorder. Summary tables will be prepared to list each MAAE, the number of subjects in each treatment group who experienced an event, and the rate of subjects with MAAE(s). All immunized subjects who complete the long term follow up will be included in the analysis.

For immunology responses, both qualitative (responder rates) and quantitative (log_{10} transformed values) results will be analyzed. Geometric mean titers will be calculated along with their 95% confidence intervals. Between groups 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 Student's t or χ^2.

Additional comparisons may be made using repeated measures analysis of variance with study group as the between subject factor and sample collection time-points as the repeated factor. Only subjects who receive at least 2 vaccine doses will be included in the immunology analysis. All statistical tests will be interpreted in a two-tailed fashion using $P < .05$ to represent statistical significance.

14.2. Planned Enrollment and Reason for 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.

14.3. Level of Significance to be Used

All statistical analysis will be made using a two-tailed alpha=0.05.

14.4. Accounting for Missing, Unused, and Spurious Data

Non-analyzable data will be documented in the deviations.

14.5. Procedures for Reporting Deviations from the Original Statistical Plan

Any deviation(s) from the original statistical plan as indicated in the protocol will be described in an amendment to the protocol and the SAP. Deviations from the SAP will be documented in accordance with NMRC SOPs.

14.6. Selection of Subjects to be Included in Analyses

All subjects who receive one or more doses of the investigational product(s) will be included in the safety analyses. Subjects who receive at least 2 doses of the investigational products will be included in the immunological analyses.

15. DIRECT ACCESS TO SOURCE DATA/DOCUMENTS

The investigator(s)/institution(s) will permit trial-related monitoring, audits, IRB review, and regulatory inspection(s) by providing direct access to source data/documents.

Subjects will be identified on eCRFs by a unique subject identification number and on source documents by name and date of birth. No personal identifier will be used in any publication or communication used to support this research study. The subject identification number will be used if it becomes necessary to identify data specific to a single subject. Representatives of PATH, the sponsor's representative, the local IRB, and the FDA are eligible to review medical and research records related to this study as a part of their responsibility to protect human subjects in clinical research. Personal identifiers will be removed from photocopied medical and research records.

15.1. Study Monitoring

Study monitoring will be the responsibility of The EMMES Corporation. Upon successful approval of the protocol and establishment of the regulatory file, the clinical monitor will establish a clinical monitoring plan. To ensure that the investigator and the study staff understand and accept their defined responsibilities, the clinical monitor will maintain regular correspondence with the site and may be present during the course of the study to verify the acceptability of the facilities, compliance with the investigational plan and relevant regulations, and the maintenance of complete records.

Monitoring visits by a sponsor's representative-designated clinical monitor will be scheduled to take place at the initiation of the study, during the study at appropriate intervals, and after the last subject has completed the study. A report of monitoring observations will be provided to the PI (for corrective actions), PATH, and the product manager.

15.2. Audits and Inspections

Authorized representatives of the sponsor, the FDA, or Institutional Review Board may visit the site to perform audits or inspections, including source data verification. The purpose of the audit or inspection is to systematically and independently examine all study-related activities and documents to determine whether these activities were conducted, and data were recorded, analyzed, and accurately reported according to the protocol, ICH GCP, and any applicable regulatory requirements.

The investigator should contact the IRB and the sponsor's representative immediately if contacted by a regulatory agency about an inspection.

15.3. Institutional Review Board (IRB)

The PI must obtain IRB approval for the study. Initial IRB approval, and all materials approved by the IRB for this study, including the subject consent form and recruitment materials, must be maintained by the investigator and made available for inspection.

The PI will be responsible for preparing and submitting continuing review reports per institution and IRB policies. The PI or a designee will submit the approved continuing review reports and the local IRB approval notifications to HRPO as soon as the documents are available. The PI or a designee will transmit the approved final study report and the local IRB approval notification to the sponsor as soon as the documents are available.

16. QUALITY CONTROL AND QUALITY ASSURANCE

To ensure compliance with GCP and all applicable regulatory requirements, the sponsor's representative may conduct quality assurance audits. Refer to section 15.2 for more details regarding the audit process.

Auditing of the clinical trial may be conducted at any time during the study to ensure continued compliance with regulations, policies and procedures. Auditing will be undertaken, as needed, by independent personnel designated by the sponsor. Audit findings will be documented in a formal audit report that will detail the conduct of the audit and summarize the observations noted.

WIRB will provide sponsor level review for PVS for this study. PVS will submit the AE/SAE to WIRB only if it is considered unanticipated and related to study product(s) according to the WIRB defined procedures.

17. ETHICS

17.1. Ethics Review

The study is based on adequately performed laboratory and animal experimentation; the study will be conducted under a protocol reviewed by Naval Medical Research Center IRB; the study is to be conducted by scientifically and medically qualified persons; the benefits of the study are in proportion to the risks; the rights and welfare of the subjects will be respected; the physicians conducting the study will ensure that the hazards do not outweigh the potential benefits; the results to be reported will be accurate; subjects will give their informed consent and will be competent to do so and not under duress; and all study staff will comply with the ethical principles in 21 CFR Part 50 and the Belmont Principles.

17.1.1. Review/Approval of Study Protocol

Before a clinical study can be initiated, the study protocol and other required documents will be submitted to the following departments for review and/or approval, with the final review by the FDA.

- NMRC Scientific Review Board
- NMRC IRB
- WIRB (Sponsor level review)
- Sponsor's Representative
- WRAIR Commander's Implementation Authorization.

Enrollment in this protocol may not begin until all approvals have been obtained and the formal authorization letter is received by the PI from the sponsor's representative.

17.1.2. Protocol Modifications

All modifications to the protocol and supporting documents (informed consent, study-specific procedures, SOPs, recruitment materials, etc.) must be reviewed and approved by the NMRC IRB prior to implementation except where necessary to eliminate apparent immediate hazards to subjects. The informed consent document must be revised to concur with any amendment as appropriate and must be reviewed and approved with the amendment. Any subject already enrolled in the study will be informed about the revision and asked to sign the revised informed consent document if the modification directly affects the individual's participation in the study. A copy of the revised, signed, and dated informed consent document will be given to the subject. All original versions of the informed consent document will be retained in the protocol regulatory file, and a copy will be retained in the clinic medical record.

17.1.3. Protocol Deviation Procedures

All subject-specific deviations from the protocol (e.g., failure to return for follow-up visits or blood collection within the time indicated in the protocol) are to be documented. The PI or designee will be responsible for identifying and reporting all deviations, which are defined as isolated occurrences involving a procedure that did not follow the study protocol or study-specific procedure. Deviations will be reported annually in the continuing review report to the

IRB, if appropriate. Action taken in response to the deviation, and the impact of the deviation will be assessed by the PI or Sub investigator.

If a protocol deviation jeopardizes the safety or rights of a subject or scientific integrity of the study, the deviation will be reported immediately to the sponsor's representative and the IRB.

17.2. Ethical Conduct of the Study

This study will be conducted in accordance with 21 CFR Part 50 and the Belmont Principles of respect for persons, beneficence, and justice.

The procedures set out in this study are designed to ensure that the sponsor's representative and all study personnel abide by the principles of the ICH GCP Guideline and the CFR. The PI confirms this by signing this study protocol and FDA Form 1572.

17.2.1. Confidentiality

In this research, the subject's health information will be collected and used to conduct the study; to monitor the subject's health status; to measure effects of the study product; to determine research results, and possibly to develop new tests, procedures, and commercial products. Health information is used to report results of research to the sponsor's representative and Federal regulators and may be reviewed during study audits for compliance with study plans, regulations, and research policies. After the study ends, each subject has the right to see and receive a copy of his/her information.

No personal identifier will be used in any publication or communication used to support this research study. The subject's identification number will be used in the event it becomes necessary to identify data specific to a single subject.

17.2.2. Compensation for Participation

All study-related medical care will be provided to subjects without cost. Should a subject be injured as a direct result of participating in this research project, s/he will be provided medical care by the staff at the Walter Reed National Military Medical Center (or other Army-affiliated medical center), at no cost to the subjects, for that injury. The subjects will not receive any injury compensation, only medical care. The subjects will not be compensated for care if s/he chooses to seek care from his/her own physician. Compensation will occur at the time of each designated visit (see Time and Event Schedule). Compensation will be provided only for completed study procedures designated for compensatory payment. Subjects will only be eligible for compensation outlined in the Informed Consent Document at the time their consent is obtained. If a subject is eligible to participate in the investigational protocol after screening, s/he will receive the following compensation:

Civilian/off-duty military compensation:

- Screening: $25
- D-7: $100
- D1: $150
- D2: $75

- D8: $125 +$25 (if subject return and complete memory aid)
- D22: $150
- D23: $50
- D29: $125 +$25 (if subject return and complete memory aid)
- D43: $150
- D44: $50
- D50: $125+$25 (if subject return and complete memory aid)
- D57: $125
- D71: $125
- D223: $25
- D403: $25
- Total: $1500

If subjects do not complete the study, their compensation will be less in proportion to the amount they did not complete.

Federal Employee/on-duty military compensation:
By regulation, active duty personnel and federal employees can be compensated only for visits in which blood draws occur, and then only $50 per visit, unless the visits occur during off-duty hours or when they are on leave. If the volunteer is off-duty or on leave, he or she will be paid the same as non-military/non-federal personnel. The total amount of compensation may vary depending on the number of visits completed.

- Screening: $25
- D-7: $50
- D1: $50
- D2: $50
- D8: $50
- D22: $50
- D23: $0
- D29: $50
- D43: $50
- D44: $0
- D50: $50
- D57: $50
- D71: $50
- D223: $0
- D403: $0
- Total: $525

Compensation for visits that are not specifically planned (scheduled) in the protocol, such as may be required to repeat labs to verify/clarify results or labs drawn to better evaluate abnormal lab values or adverse events may be compensated at the discretion of the PI relative to the severity of the event and scope of evaluations. For unplanned visits for active duty subjects, compensation limitations as previously described will apply. Civilian and military subjects who have completed the screening process for this study will be given the opportunity

to receive compensation for assisting in recruitment of additional subjects. Subjects will be asked to direct interested persons to contact the CTC. Subjects will receive $25 for each referred person who then attends a screening session and meets all inclusion and none of the exclusion criteria. Compensation will be independent of the referred person's decision to enroll. Referral compensation for active duty personnel and federal employees is subject to the same restrictions described earlier. Final authority over dispensation of referral compensation will lie with the CTC director. Subjects asked to come to the clinic as an alternate on the first vaccination day of a cohort may be compensated $50 if they are not enrolled in that cohort.

17.2.3. Medical Care for Research-Related Injuries

If a subject is injured because of participation in this research and is a DoD healthcare beneficiary (e.g., active duty in the military, military spouse or dependent), the subject is entitled to medical care for that injury within the DoD healthcare system, as long as the subject remains a DoD healthcare beneficiary. This care includes, but is not limited to, free medical care at Army hospitals or clinics.

If a subject is injured because of participation in this research and is not a DoD healthcare beneficiary, the subject is entitled to free medical care for that injury at an Army hospital or clinic. It cannot be determined in advance which Army hospital or clinic will provide care. If the subject receives care for research-related injuries outside of an Army hospital or clinic, the subject or the subject’s medical insurance will be responsible for medical expenses.

For all subjects: Transportation to and from Army hospitals or clinics will not be provided. No reimbursement is available if the subject incurs medical expenses to treat research-related injuries. No compensation is available for research-related injuries. The subject is not waiving any legal rights. The subject should contact the PI if the subject believes he or she has sustained a research-related injury. The subject should contact the PI for any questions.

Requests for other benefits, such as compensation for lost time from work, are processed independently of this protocol. Military members retain the right to pursue military disability benefits, and Federal civilian employees retain the right to pursue relief through established workers compensation processes, but neither military disability benefits nor workers compensation benefits are guaranteed. The right of other parties to seek redress against the United States Government is limited to that set forth by existing agency regulations and the Federal Tort Claims Act. The subject should understand that this does not constitute a waiver or release of legal rights. This issue is addressed in the informed consent and will be discussed with the subject by the investigator or designee before the subject signs the informed consent to participate in the study.

17.3. Written Informed Consent

The informed consent process and document will be reviewed and approved by the IRB and sponsor’s representative prior to initiation of the study. The consent document contains a full explanation of the possible risks, advantages, and alternate treatment options, and availability of treatment in the case of injury, in accordance with 21 CFR 50. The consent document indicates that by signature, the subject, or where appropriate, legal guardian, permits access to relevant

medical records by the sponsor's representative and by representatives of the FDA. The sponsor's representative will submit a copy of the initial IRB- and sponsor's representative-approved consent form to the FDA and will maintain copies of revised consent documents that have been reviewed and approved by the IRB/ethics committee.

A written informed consent document, in compliance with 21 CFR Part 50, 32 CFR Part 219, and the Belmont Principles will be signed by the subject before any study-related procedures are initiated for that subject. This consent document must be retained by the investigator as part of the study records. The investigators or their designees will present the protocol in lay terms to individual subjects. Questions on the purpose of the protocol, protocol procedures, and risks to the subjects will then be solicited. Any question that cannot be answered will be referred to the PI. The subject will be allowed to take the consent document home to consider and discuss it with others and return to the CTC at a later time to sign it. The subject should understand that the study product is an investigational drug and is not licensed by the FDA for commercial use, but is permitted to be used in this clinical research. Informed consent includes the principle that it is critical the subject be informed about the principal potential risks and benefits. This information will allow the subject to make a personal risk versus benefit decision and understand the following:

- Participation is entirely voluntary,
- Subjects may withdraw from participation at any time,
- Refusal to participate involves no penalty, and
- The individual is free to ask any questions that will allow him/her to understand the nature of the protocol.

A description of this clinical trial will be available on http://www.ClinicalTrials.gov, as required by US law.

Should the protocol be modified, the subject consent document must be revised to reflect the changes to the protocol. If a previously enrolled subject is directly affected by the change, the subject will receive a copy of the revised informed consent document. The approved revision will be read, signed, and dated by the subject.

Military personnel will be recruited by CTC personnel. No recruitment of military personnel will occur in the presence of his/her supervisor. There is no benefit to military personnel for participating in this study. There will be no coercion or disciplinary actions for not participating or withdrawing if enrolled.

18. DATA HANDLING AND RECORDKEEPING

The primary source document for this study will be the subject's record. If separate research records are maintained by the investigator(s), the medical record and the research records will be considered the source documents for the purposes of auditing the study. The source documents will be retained at the site.

For this study, an EDC database system will be used for the collection of the study data in an electronic format. The EDC database system will be designed based on the protocol requirements, the approved eCRF layouts and specifications, and in accordance with 21 CRF Part 11. The eCRF layouts and specifications define and identify the applicable source data that will be collected and captured into the EDC database system. The applicable source data will be electronically transcribed by the site designee onto the eCRF (data entry screens) in the EDC database system. The investigator is ultimately responsible for the accuracy of the data transcribed on the eCRF. Data monitoring and management will be performed in the EDC database system by the study monitor and the designated Data Management group.

A detailed data management plan will be written and approved by the study team and the PI prior to study start. All updates to the data management plan must be approved before study close-out and database lock.

18.1. Inspection of Records

The sponsor’s representative or designee will be allowed to conduct site visits to the investigation facilities for the purpose of monitoring any aspect of the study. The investigator agrees to allow the monitor to inspect the drug storage area, investigational product stocks, drug accountability records, subject charts, study source documents, and other records relative to study conduct.

Subjects’ health information is used to report results of research to the sponsor’s representative and Federal regulators and may be reviewed during study audits for compliance with study plans, regulations, and research policies. The consent document indicates that by signature, the subject permits access to relevant medical records by the sponsor’s representative and by representatives of the FDA.

Upon a subject’s termination from the trial, completed eCRFs will be ready and available for on-site review by the sponsor’s representative or the designated representative within 14 days of receipt of study data.

18.2. Retention of Records

The PI will maintain all documentation relating to the study for a period of 2 years after the date a marketing application is approved for the investigational product or for 2 years following the discontinuance of the investigational product for investigation. The records will be kept in a secure area with access by authorized personnel only. If it becomes necessary for the sponsor’s representative or designee or the FDA to review any documentation relating to the study, the investigator must permit access to such records.

Completed, monitored eCRFs will be stored in a secure location by the sponsor's representative or designee. A copy of each completed eCRF will be retained by the investigator.

The PI will be responsible for retaining sufficient information about each subject, i.e., name, address, telephone number, and subject identifier in the study, so that the sponsor's representative, the local IRB, the FDA or other regulatory authorities may have access to this information should the need arise.

19. PUBLICATION POLICY

All data collected during this study will be used to support this IND. All publications and presentations are governed by the standards and norms detailed in NAVMEDRSCHCENINST 5721.1. All authors will submit the proposed publication/presentation at least 30 days prior to the submission date. Prior to submission, the directorate will conduct a substantive scientific and professional review. The document is routed to the Office of Research Administration (ORA) for review and routing for Command review and approval, ultimately by the NMRC Public Affairs Officer (PAO). Once it is cleared at NMRC, it will be forwarded to BUMED through NMSC, if appropriate. Prior to publication, an author must have a completed Publication Clearance Request Submission Form with signatures from all approving and reviewing authorities.

20. LIST OF REFERENCES

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