A Phase 1 Double-Blinded, Placebo-Controlled, Dose Escalation Study to Evaluate the Safety and Immunogenicity of Double Mutant Heat-Labile Toxin LTR192G/L211A (dmLT) from Enterotoxigenic *Escherichia coli* (ETEC) by Oral, Sublingual, or Intradermal Vaccination in Adults Residing in an Endemic Area

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Version Number: 5.0

06 November 2019

STATEMENT OF COMPLIANCE

This trial will be conducted in accordance with Good Clinical Practices (GCP) as required by the following:

- United States Code of Federal Regulations (CFR) applicable to clinical studies (45 CFR Part 46, 21 CFR Part 50, 21 CFR Part 54, 21 CFR Part 56, and 21 CFR Part 312);
- International Conference on Harmonization (ICH) E6; 62 Federal Register 25691 (1997);
- U.S. National Institutes of Health (NIH) Clinical Terms of Award, as applicable;
- And the local laws and regulations of Bangladesh and International Centre for Diarrheal Disease Research, Bangladesh (icddr,b).

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

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

SIGNATURE PAGE

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

Site Investigator:

Signed: _____ Date: _____

Wilbur H. Chen, MD, MS
Protocol Principal Investigator

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

AE	Adverse Event/Adverse Experience
ALS	Antibodies in Lymphocyte Supernatant
ALT	Alanine Aminotransferase, formerly called SGPT
ANC	Absolute Neutrophil Count
ASC	Antibody Secreting Cells
AST	Aspartate Aminotransferase, formerly called SGOT
cAMP	Cyclic Adenosine Monophosphate
CFR	Code of Federal Regulations
cGMP	Current Good Manufacturing Practice
CI	Confidence Interval
CfaE	CFA/I minor pilin subunit
CPM	Clinical Project Manager
CFR	Code of Federal Regulations
CRF	Case Report Form
CT	Cholera Toxin
CTL	Cytotoxic T Cell
CVD	Center for Vaccine Development-Global Health
CyTOF	Cytometry by Time-of-Flight, or Mass Cytometry
DHHS	Department of Health and Human Services
DMID	Division of Microbiology and Infectious Diseases, NIAID, NIH, DHHS
dmLT	Double-Mutant Heat-Labile Toxin
DSMB	Data and Safety Monitoring Board
eCRF	Electronic Case Report Form
EHDB	Enteric and Hepatic Disease Branch, DMID, NIAID, NIH, DHHS
ELISA	Enzyme-Linked Immunosorbent Assay
ELISpot	Enzyme-Linked ImmunoSpot Assay
ETEC	Enterotoxigenic *Escherichia coli*
EVI	Enteric Vaccine Initiative, of PATH
FDA	Food and Drug Administration, DHHS
FWA	Federal-wide Assurance
GEMS	Global Enteric Multicenter Study
GCP	Good Clinical Practice
HBsAg	Hepatitis B Virus Surface Antigen
HCV	Hepatitis C Virus
HEENT	Head, Eyes, Ears, Nose, Throat

Hg	Hemoglobin
HIPAA	Health Insurance Portability and Accountability Act
IB	Investigator's Brochure
icddr,b	International Centre for Diarrhoeal Disease Research, Bangladesh
ICF	Informed Consent Form
ICH	International Conference on Harmonisation
ID	Intradermal
IEC	Independent or Institutional Ethics Committee
Ig	Immunoglobulin
IM	Intramuscular
IND	Investigational New Drug Application
IRB	Institutional Review Board
ISM	Independent Safety Monitor
IVF	Intravenous Fluids
LT	Labile Toxin, or *E. coli* Heat-Labile Enterotoxin
MedDRA®	Medical Dictionary for Regulatory Activities
mLT	Single mutant Heat-Labile Toxin
MM	Medical Monitor
MO	Medical Officer
MOP	Manual of Procedures
MSD	moderate-to-severe diarrhea
NIAID	National Institute of Allergy and Infectious Diseases, NIH, DHHS
NIH	National Institutes of Health
OCRA	Office of Clinical Research Affairs, DMID, NIAID, NIH, DHHS
OHRP	Office for Human Research Protections, OASH, OS, DHHS
ORA	Office of Regulatory Affairs, DMID, NIAID, NIH, DHHS
ORS	Oral Rehydration Solution
OVA	Ovalbumin Antigen
PATH	Program for Appropriate Technology in Health, now simply "PATH"
PBMC	Peripheral Blood Mononuclear Cells
PBS	Phosphate Buffered Saline
PHI	Personal Health Information
PI	Principal Investigator
PK	Pharmacokinetics
QA	Quality Assurance
QC	Quality Control
QMP	Quality Management Plan
SAE	Serious Adverse Event/Serious Adverse Experience

SDCC	Statistical and Data Coordinating Center
SDS-PAGE	Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis
SL	Sublingual
SMC	Safety Monitoring Committee
SOP	Standard Operating Procedure
ST	Stable Toxin, or *E. coli* Heat-Stable Enterotoxin
TCI	Transcutaneous Immunization
TD	Traveler's Diarrhea
TT	Tetanus Toxoid
UMB	University of Maryland, Baltimore
US	United States
USAMRIID	US Army Medical Research Institute of Infectious Diseases
USP	United States Pharmacopeia
VTEU	Vaccine and Treatment Evaluation Unit
WBC	White Blood Cell
WFI	Water For Injection
WHO	World Health Organization
WRAIR	Walter Reed Army Institute of Research

PROTOCOL SUMMARY

Title:	A Phase 1 Double-Blinded, Placebo-Controlled, Dose Escalation Study to Evaluate the Safety and Immunogenicity of Double Mutant Heat-Labile Toxin LTR192G/L211A (dmLT) from Enterotoxigenic *Escherichia coli* (ETEC) by Oral, Sublingual, or Intradermal Vaccination in Adults Residing in an Endemic Area
Phase:	1
Population:	Approximately 135 Healthy Adults, ages 18 to 45 years
Number of Sites:	Single site, Mirpur, Bangladesh
Study Duration:	Approximately 2.5 years, including 6 months of follow up for the last cohort
Subject Participation Duration:	Up to 9 months
Description of Agent or Intervention:	Attenuated, Recombinant Double Mutant Heat-Labile Toxin LTR192G/L211A (dmLT) from Enterotoxigenic *Escherichia coli* (ETEC)
Study Objectives:	Primary: • To assess the reactogenicity, safety and tolerability of dmLT when administered in three sequential doses, over a range of dosages by oral, sublingual, or intradermal routes Secondary: • To assess the long-term safety, from first vaccination through 6 months following the last dose of vaccine • To evaluate the serum anti-dmLT IgG and IgA response • To evaluate the IgG and IgA anti-dmLT Antibody Secreting Cell (ASC) response

- To evaluate the IgG and IgA anti-dmLT Antibodies in Lymphocyte Supernatant (ALS) response
- To evaluate the total fecal IgA and fecal anti-dmLT IgA response
- To evaluate the total salivary IgA and the saliva-derived anti-dmLT IgA response

Exploratory:

- To measure the mucosal homing of IgA anti-dmLT ASC
- To measure the serum toxin neutralizing antibody response
- To measure the dmLT-specific IgG and IgA memory B cell response
- To determine the dmLT-specific effector and memory T cell responses

Study Outcome Measures:

Primary Safety Endpoints:

- The occurrence of solicited local site and systemic reactogenicity events from vaccination through 7 days after each dose of vaccine is administered
- The occurrence of study withdrawal throughout the study
- The occurrence of discontinuation of study vaccination during the study
- The occurrence of unsolicited vaccine-related adverse events (AE), including laboratory AE, from first vaccination through 28 days after the last dose of vaccine is administered

Secondary Safety Endpoints:

- The occurrence of vaccine-related serious adverse events (SAE) from the first vaccination through 6 months after the last dose of vaccine is administered

Secondary Immunogenicity Endpoints:

- At any time after vaccination, the proportion of participants with a ≥4-fold rise in dmLT-specific serum IgG and IgA titers over baseline measured by ELISA
- At any time after vaccination the proportion of participants with >8 dmLT-specific IgA or IgG ASC / 10^6 PBMC as measured by ELISpot
- At any time after vaccination, the proportion of participants with ≥2-fold rise in ALS anti-dmLT-specific IgG and IgA titers over baseline measured by ELISA
- At any time after vaccination, the proportion of participants with a ≥4-fold rise over baseline in dmLT-specific fecal IgA titers measured by ELISA
- At any time after vaccination, the proportion of participants with a ≥4-fold rise over baseline in dmLT-specific salivary IgA titers measured by ELISA

Exploratory Immunogenicity Endpoints:

- Geometric mean titers of dmLT-specific serum IgG and IgA measured by ELISA at time points listed in Appendix A
- Mean and median number of dmLT-specific IgG and IgA ASC measured by EliSpot at time points listed in Appendix A
- Geometric mean titers of dmLT-specific IgG and IgA in ALS measured by ELISA at time points listed in Appendix A
- Geometric mean titers of dmLT-specific fecal IgA measured by ELISA at time points listed in Appendix A
- Geometric mean titers of dmLT-specific salivary IgA measured by ELISA at time points listed in Appendix A
- The proportion of participants with dmLT-specific memory B cell response as measured by ELISpot at time points listed in Appendix A
- At any time after vaccination, the proportion of participants with anti-dmLT IgG and IgA ASC in circulation expressing gut homing receptors (integrin

α4β7 in the absence or presence of CD62L) measured by EliSpot assay at time points listed in appendix A

- At any time after vaccination, proportion of participants with ≥4-fold rise over baseline in toxin neutralization titers measured by Y-1 cell assay
- At any time after vaccination, the proportion of participants with dmLT-specific effector and memory T cell responses as measured by CyTOF at time points listed in appendix A

Description of Study Design:	This is a phase 1 double-blinded, placebo-controlled, dose-escalation study which will evaluate the safety and immune responses to a range of dosages of dmLT, when administered by the oral, sublingual, or intradermal route. Two independent Data and Safety Monitoring Boards (DSMBs) will monitor this study. One, local DSMB will be convened by and advisory to the icddr,b Ethical Review Committee (ERC). The other will be a NIH DSMB that is convened and advisory to DMID. See Section 9.6 Safety Oversight, for a description of the safety oversight to be conducted between the 2 independent review boards.
Estimated Time to Complete Enrollment:	Approximately 20 months

Table 1: Schematic of Study Design:

Cohort	Group	Route	dmLT dose (µg)	Target No. Participant Enrollment	Minimum No. Participants Evaluable*	Vaccination Days
A	A1 A2	Oral	5 placebo	12 3	10 1	1, 15, 29
ISM, MM, and the protocol PI will review Cohort A safety data collected through 7-days post vaccination of third dose of last participant for recommendation to proceed to the cohort B.						
B	B1 B2	Oral	25 placebo	12 3	10 1	1, 15, 29
C	C1 C2	Sublingual	5 placebo	12 3	10 1	1, 15, 29
ISM, MM, and the protocol PI will review Cohort C safety data collected 7-days post vaccination of third dose of last participant for recommendation to proceed to the cohort D.						
D	D1 D2	Sublingual	25 placebo	12 3	10 1	1, 15, 29
E	E1 E2	Intradermal	0.3 placebo	12 3	10 1	1, 22, 43
ISM, MM, and the protocol PI will review Cohort E safety data collected through 7-days post vaccination of third dose of last participant for recommendation to proceed to the cohort F.						
F	F1 F2	Intradermal	1.0 placebo	12 3	10 1	1, 22, 43
Scheduled DMID DSMB meeting will be held to review cumulative safety data through 7 days after the third dose of vaccine and determine if study should proceed to Cohorts G, H and I.						
G	G1 G2	Oral	50 placebo	12 3	10 1	1, 15, 29
H	H1 H2	Sublingual	50 placebo	12 3	10 1	1, 15, 29
I	I1 I2	Intradermal	2.0 placebo	12 3	10 1	1, 22, 43
Scheduled DMID DSMB meeting will be held to review the aggregate safety data through 28 days after the third dose of vaccine for all nine cohorts (A-I)						
A Final DMID DSMB meeting will be held to review the safety data following the completion of the study						

Note: the local DSMB will conduct a review of each cohort (A-I) by review of safety data through 7 days after the third dose of vaccine for each cohort. (see Section 9.6.3) The local DSMB will also be provided the final safety report, the same report that will be generated for the final DMID DSMB meeting.

* Evaluable participants are defined as completing all 3 doses of vaccine and providing both safety information and protocol-required specimens through at least 7 days after the third dose of

vaccine. Participants receiving at least one dose of the investigational product must be followed for safety until the end of the study. If additional participants are required, to maintain blinding, additional participants are to be determined by Emmes; to keep the blind, at least one placebo will be included.

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2 BACKGROUND INFORMATION AND SCIENTIFIC RATIONALE

2.1 Background Information

Enterotoxigenic *Escherichia coli* (ETEC), one of six recognized diarrheagenic *E. coli*, is a mucosal non-invasive bacterial enteropathogen that causes acute watery diarrhea. The Center for Vaccine Development (CVD)'s recent Global Enteric Multicenter Study (GEMS) confirmed ETEC as one of the top 5 attributable pathogens responsible for causing moderate-to-severe diarrhea (MSD) among infants and young children of developing countries.[1] ETEC is also the most common cause of diarrhea for persons from industrialized countries who visit developing countries. ETEC is estimated to cause approximately 400 million diarrheal episodes and 380,000 deaths globally each year (WHO 2009). Nonetheless, despite the public health importance, there is no licensed vaccine to prevent ETEC.

Heat-labile Toxin or LT

ETEC strains produce a highly immunogenic heat-labile toxin (LT) and/or a small (18-19 amino acid polypeptide), poorly immunogenic heat-stable toxin (ST). LT has been more extensively studied as a potential vaccine antigen. The LT protein, which structurally and functionally acts like cholera toxin (CT), is a multimeric (1 A-subunit: 5 B-subunits) enzyme that catalyzes ADP ribosylation of GTP binding proteins within polarized intestinal epithelial cells. The A-subunit is the toxic enzymatic moiety while the B-subunits facilitate toxin uptake by binding GM1 and other galactose-containing cell-surface components. The result of A-subunit intoxication is up-regulation of intracellular cAMP leading to hypersecretion of water and electrolytes in the small intestine, resulting in diarrhea and potentially severe dehydration. LT is also believed to serve as an accessory colonization factor for ETEC strains, in that, LT expressing strains colonize better and are more virulent in animal models.[2-4] In GEMS, ST only and ST plus LT producing ETEC were associated with MSD but LT-only ETEC strains were not.

As a principal virulence factor of ETEC, LT has been studied as a potential vaccine antigen[5] and has been shown to confer short term protection in animal models and in limited human field trials.[6-8] An ETEC vaccine candidate based on native LT delivered by transcutaneous immunization (TCI) with a dermal patch was found to be immunogenic, and demonstrated some efficacy in Phase 2b and Phase 3 field efficacy trials in travelers to Guatemala and Mexico.[8,9] In addition, recent field studies of cholera vaccines which are capable of inducing cross-reactive anti-LT toxin immunity also indicate that an anti-LT based vaccine can be protective at least in travelers against ETEC and that anti-LT vaccines may provide protection against a broader array of ETEC pathotypes than originally anticipated.[10-12]

LT or its derivatives have also been extensively studied as a robust immunostimulating adjuvant to vaccines.[13,14] Thus, the LT of ETEC is a unique protein with the potential to be both a stand-alone vaccine as well as a mucosal adjuvant for other co-administered vaccine antigens. The adjuvanting property of this protein may be particularly relevant for use in ETEC or other enteric vaccine candidates or combination vaccines since enteric vaccines have historically been poorly immunogenic when given to infants and young children in developing countries.[15] Consequently the inclusion of a non-toxic mutant of the LT protein in future enteric vaccines formulations would not only help protect vaccinees against ETEC but might serve to substantially improved the antibody and cellular responses to other ETEC, *Shigella*, Cholera, Rotavirus or Typhoid antigens administered along with it.[5,14]

However, native LT has considerable enterotoxicity for humans, thereby limiting its use as an adjuvant. For example, in a study conducted at the CVD and at Lausanne University Hospital, oral doses of 5 and 10 µg of native LT, administered with a recombinant *Helicobacter pylori* urease protein, elicited diarrhea among 67% (16 of 24).[16]In a subsequent study of lower native LT doses, administered with a *H. pylori* urease vaccine, mild diarrhea (1-4 loose stools) occurred in 50% (6 of 12) of volunteers exposed to 2.5 µg of native LT.[17]

Single-mutant LT (LTR192G) or mLT

To render LT safe for human use, site-directed mutagenesis was performed to eliminate the ADP-ribosylating enzymatic activity of the A subunit while maintaining the immunogenic B subunit. A single mutant (mLT) LTR192G was constructed by substituting glycine for arginine at position 192 within the disulfide subtended region of the A subunit, separating the A1 from A2.[18]The US Army Medical Research Institute of Infectious Diseases (USAMRIID) conducted a randomized, placebo-controlled, dose-escalation study in 36 healthy adult subjects who received a single oral dose of mLT (5, 25, 50, or 100 µg) or placebo. An oral dose of mLT at 100 µg elicited severe diarrhea (>1L within 24 h) among 2 of 12 recipients.[19] When mLT was orally administered at the CVD, in the context of an adjuvant to an inactivated whole-cell *H. pylori* vaccine, 5 of 34 (16%) subjects who received 25 µg of mLT developed diarrhea (3-17 stools over 1-3 days). One of 3 subjects given 25 µg of mLT alone also developed diarrhea (33%).[20] Mild-to-moderate diarrhea was also observed with a *Campylobacter* whole-cell vaccine adjuvanted with mLT. Nine of 39 subjects (23%) developed mild-to-moderate diarrhea within 24-36 hours after receiving their first dose of the vaccine plus 25 µg mLT adjuvant combination.[21] Whereas, in a study of a microencapsulated *E. coli* surface antigen C6 (meCS6), 60 subjects received meCS6 with or without 2 µg of mLT (significantly lower dose than in previous studies) and there was no difference in the rates of diarrhea and limited adjuvanticity observed.[22]

In a phase 1 study comparing the safety and immunogenicity of fimbriae CFA/I minor pilin subunit E (CfaE) tip-adhesion protein with 100 ng of mLT via transcutaneous (TCI) and intradermal (ID) routes, the ID route was shown to be superior to TCI in inducing both serum and mucosal responses to the mLT adjuvanted CfaE candidate vaccine. Tenderness and pain was more commonly reported with ID compared to TCI vaccination but was generally mild and brief. There were no clear systemic adverse events attributable to the ID administration of vaccine. Vaccine site reactions (rash) and pruritus were common but the severity was generally mild and resolved in all subjects. Six months after receiving the first vaccine dose, 5 subjects (10.2%) reported a persistent vaccine site reaction. Additionally, persistent skin color changes were reported by 22 subjects 44.9%) that included hypopigmentation (2 subjects; 4.1%) and hyperpigmentation (20 subjects; 40.8%). The frequency of skin pigmentation changes decreased over the following six months with only 9 subjects (18.4%) reporting persistent hyper-(3 subjects; 4.1%) or hypopigmentation (6 subjects; 12.2%) 1 year following the first vaccination. (Unpublished data from S. Savarino, NMRC; NCT01644565)

Based on the tolerable safety profile and superior immunogenicity results (unpublished data), it was determined that the ID administered 25 µg of CfaE with mLT would be utilized in a Phase 2b challenge study as a proof-of-concept for parenteral administration of an ETEC antigen (unpublished data; NCT01922856). The vaccination and challenge phases have completed and similar to the phase 1 trial, among the 56 vaccine recipients, there were no vaccine-attributable systemic adverse events while vaccine site reactions (rash) and pruritus were common. Subjects are currently undergoing long-term safety follow-up at the 6- and 12-month post vaccination time points.

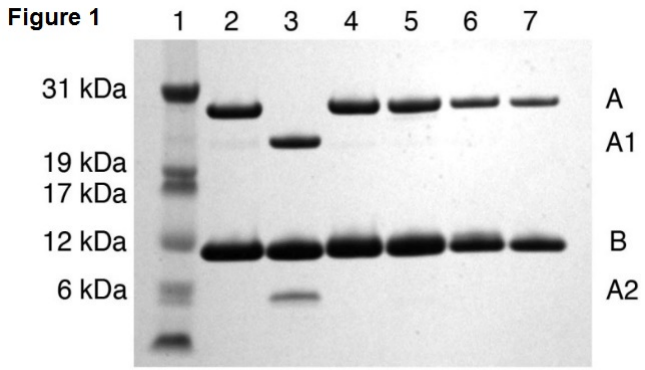
Derivation and Characterization of double-mutant LT (R192G/L211A) or dmLT

A second-generation derivative, double-mutant LT (R192G/L211A) or dmLT, was constructed by a further substitution of leucine for alanine at amino acid position 211.[23]This substitution was initially believed to be a putative pepsin-sensitive proteolytic cleavage site, but there is no evidence that LT is cleaved by pepsin either *in vitro* or *in vivo* (data not shown), so the biochemical basis of attenuation is unclear.

dmLT has been characterized in four ways: a) sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE, Figure 1), b) in vitro cAMP production (Figure 2), c) enterotoxicity in mice (Figure 3), and d) immunogenic and adjuvant capacities in mice (Figure 4A and B). These structural, biological, and immunogenic analyses confirmed that this material is in fact dmLT; that native LT-like molecules retaining the trypsin sensitivity and cAMP inducing activity of the wild-type holotoxin are not present; that dmLT lacks enterotoxicity at the doses tested in mice, and that the mutant retains the ability to induce immune response to itself as well as adjuvant the immune response to a model protein antigen, like tetanus toxoid (TT). SDS-

PAGE was used to confirm that, unlike the native LT holotoxin, the A subunit of dmLT was not susceptible to trypsin cleavage. In vitro cAMP induction and in vivo patent mouse assays were used to determine the extent to which dmLT may have reduced enzymatic or enterotoxic activity compared to the wild-type toxin, while oral immunization of mice was used to demonstrate that the mutant retains its ability to serve as both an antigen and adjuvant.

Figure 1: **SDS-PAGE gel of native LT, single mutant LT and double mutant LT**



SDS-PAGE gel of 10 µg of native LT (lanes 2 and 3), LTR192G (lanes 4 and 5), or LT(R192G/L211A) Lot No. 1575 with (lane 7) or without (lane 6) treatment with 200 ng of trypsin for 60 min at 37ºC. This assay demonstrates trypsin cleavage (activation) of native LT (lane 3) and confirms the absence of native LT-like molecules in the LTR192G and LT (R192G/L211A; Lot No 1575) preparations. A comparison of lanes 6 and 7 confirms the resistance of Lot No. 1575 to trypsin digestion (lane 7) and confirms the absence of any native LT in Lot No. 1575 (comparing lane 3 with lane 7). SDS-PAGE analysis of trypsin treated and untreated preparations: lane 1, molecular weight markers; lane 2, native LT; lane 3, native LT treated with trypsin; lane 4, LTR192G; lane 5, LTR192G treated with trypsin; lane 6, LT(R192G/L211A); lane 7, LT(R192G/L211A) treated with trypsin. (Unpublished data, from current Investigator's Brochure).

Figure 2: Enzymatic activity of native LT, single mutant LT and double mutant LT

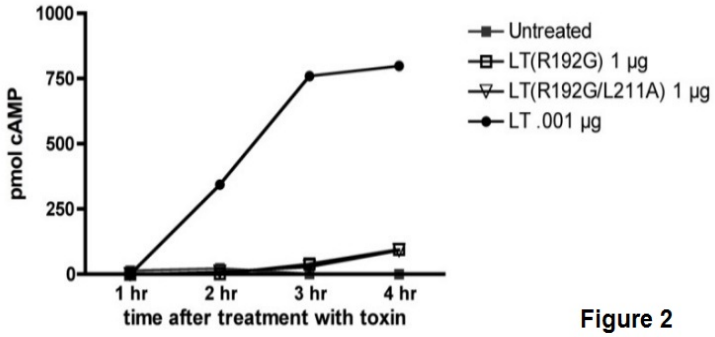
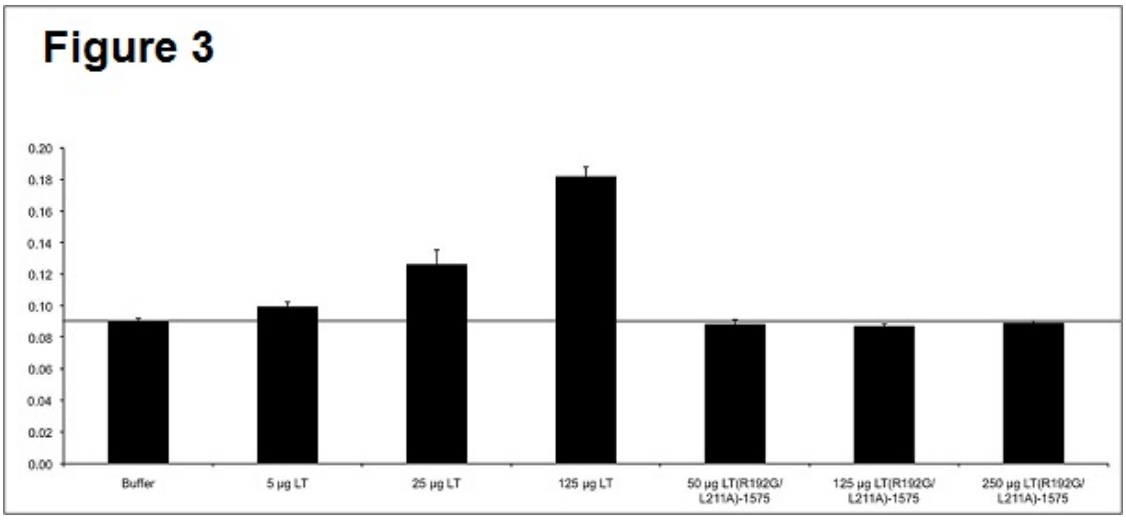


Figure 2

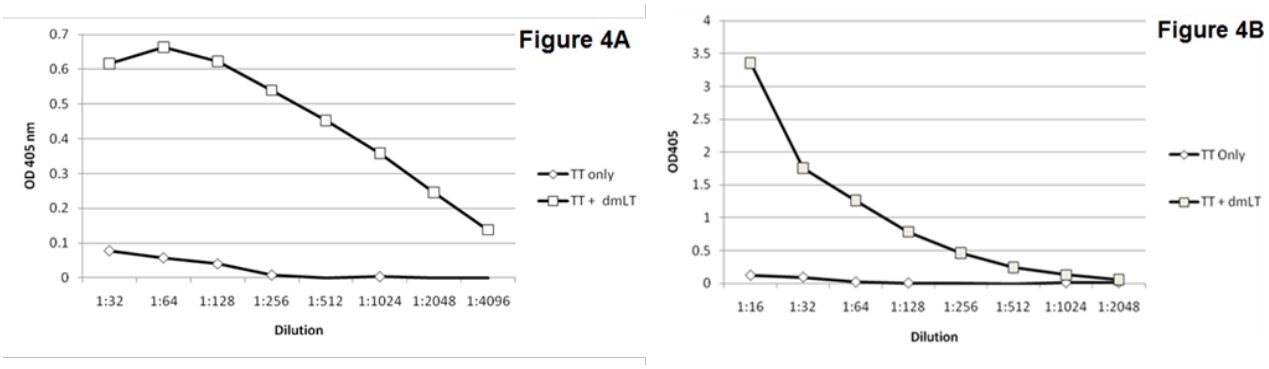
The enzymatic activity of LT, LT(R192G) and LT(R192G/L211A) based on cAMP induction (accumulation) as measured in cultured Caco-2 cells. Dilutions of trypsin-treated native LT were added to one set of wells to achieve a final concentration of 0.001 µg of native LT per well. One microgram of trypsin-treated LTR192G or LT(R192G/L211A) was added to another set of wells. At 1, 2, 3, and 4 hours after toxin addition, cells were washed twice with cold phosphate buffered saline (PBS). Intracellular cAMP was extracted by adding 0.4 mL of 0.1 N HCl to each well and incubated at room temperature for 20 minutes. cAMP was detected using an ELISA-based low pH cAMP kit (Figure 2). The inability of 1 µg of LT(R192G/L211A) to elicit as much intracellular cAMP as 0.001 µg of native LT indicates that this mutant enterotoxin is attenuated in enzymatic activity in comparison to native LT (Unpublished data from J.D. Clements, Tulane).

Figure 3: Comparative Enterotoxicity of native LT, single mutant LT and double mutant LT in mice.



The comparative enterotoxicity of native LT and LTR192G/L211A (Lot No. 1575) in the Patent Mouse Model. For the patent mouse assay, four female BALB/c mice per group were inoculated with 0.5 mL of buffer; 5, 25, and 125 µg native LT; or 50, 125, and 250 µg LT(R192G/L211A) Lot No. 1575. Inoculations were made intragastrically with a blunt-tip feeding needle. Following inoculation, animals remained in their cages without food but with water ad libitum for 3 hours and were then sacrificed. The entire intestine from duodenum to anus from each mouse was removed carefully to retain any accumulated fluid; residual mesentery was eliminated before weighing. The carcass was weighed separately and a gut:carcass ratio (Y axis Figure 3) was calculated for each animal. Mean gut:carcass ratio for 50 µg of LT(R192G/L211A) should be less than that obtained with 5 µg of native LT. As shown in Figure 4, the reduced enterotoxicity of LT(R192G/L211A) was clearly evident since 250 µg of Lot No. 1575 elicited no more fluid than the buffer control and the gut:carcass ratio was less than 5 µg of native LT at any of the dilutions tested (Unpublished data, from Investigator's Brochure).

Figure 4: Oral antigenicity and Adjuvanticity of dmLT (Lot 1575)

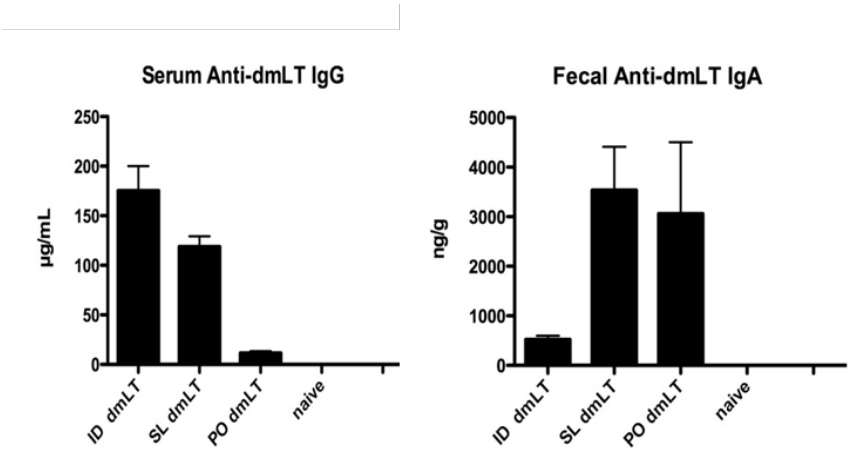


Animals that were orally immunized with LT(R192G/L211A) developed a significant serum anti-LT IgG response (Figure 4A) confirming the oral antigenicity of LT(R192G/L211A). The adjuvanticity of LT (R192G/L211A) was monitored biologically in an oral adjuvanticity assay using TT (Statens Serum Institute, Denmark) as a test antigen. Groups of 10 BALB/c mice were immunized orally with a blunt-tip feeding needle, once per week for 3 weeks, with either 100 µg of TT alone or with the same dose of TT admixed with 25 µg of LT(R192G/L211A). One week after the third immunization, serum anti-TT was determined by ELISA, and the adjuvant activity of Lot No. 1575 was highly significant in this assay. As seen in Figure 4B, LT(R192G/L211A) significantly increased the serum anti-TT IgG response in orally immunized animals compared to the response in animals receiving TT alone. This assay confirms the oral adjuvanticity of LT(R192G/L211A). (Unpublished data, from current Investigator's Brochure)

Sublingual and Intradermal dmLT

Recent animal studies have demonstrated that the sublingual (SL) route of administration elicits serum (IgG) and local intestinal fecal (IgA) antibodies to vaccine antigens that are comparable or better than those induced by the intradermal (ID) and oral (per os [PO]) routes. These observations may be particularly relevant for enteric vaccines. As shown in the Figure 5, the magnitude of fecal anti-dmLT IgA response was better in those animals receiving dmLT by the SL versus ID route, which further suggests that the SL route may be better for inducing local intestinal anti-dmLT antibodies than the ID or oral route of delivery (Unpublished data from J.D. Clements, Tulane).

Figure 5: Magnitude of serum IgG and fecal IgA dmLT-specific responses to different routes of administration



Other animal studies have shown that the SL and ID routes are not only well tolerated, but induce mucosal immune responses on a broad range of mucosal surfaces, including the respiratory, gastrointestinal, and urogenital tracts.[14] Chemotactic responses of IgG and IgA antibody secreting cells (ASCs) after SL administration of 2 µg of CT showed that CCL28 (a β-chemokine that possesses2 or more adjacent cysteines) plays a critical role in the selective homing of IgA ASCs into genital tissues as well as other mucosal sites.[24] SL administration of a combination CT and live influenza virus at vaccine doses that would be lethal by the nasal route were well tolerated (no significant histopathology noted) and highly immunogenic in mice.[25] Administration of non-replicating antigens (ovalbumin antigen [OVA]),[25] *Bacillus subtilis*-expressing TT fragment C,[26-28] inactivated whole cell Pneumococcal vaccine,[29] *Helicobacter pylori*,[20] and live influenza virus,[24] some in the presence of CT or mutant LT proteins (mLT and dmLT), has been shown to induce broad-based systemic and mucosal immune responses in the targeted mucosal area. In 3 of the 5 above mentioned vaccinations via the SL route, subsequent challenge with the respective wild type strains of human papilloma virus (HPV),[30] *Helicobacter pylori*,[31] and *Streptococcus pneumoniae*[29] protection was conferred and strong antigen-specific serum IgG and IgA responses in the targeted mucosal tissues were observed.

Accumulating preclinical data continues to indicate that the SL route is a very robust and encouraging new option for effective mucosal immunization; however, the utility (safety and efficacy) of this route for immunizing humans has yet to be established.[32] As a result, studies exploring the SL route are pivotal for the mucosal vaccine development field as another option for effective active mucosal immunization with protein antigens like dmLT. The significant

attenuation of dmLT is expected to further eliminate the possibility of facial palsy, which has been observed with native LT when delivered by the intranasal route.[33]

Buoyed by these positive results, multiple enteric vaccine studies are being planned for the use of dmLT as an adjuvant (private communication with PATH-Enteric Vaccine Initiative). Significant questions remain to be addressed in future studies. First, how will dmLT perform (through the assessment of safety and immunogenicity) in an endemic country, when compared to studies performed in an industrialized country? Second, are there significant differences in the responses by the route of administration (oral, SL, or ID)?

2.1.1 Background on the Study Sites

Center for Vaccine Development-Global Health (CVD) - Baltimore, Maryland, U.S.A.

The CVD has been an NIAID Vaccine and Treatment Evaluation Unit (VTEU) for ~40 years and has a formidable track record of research on vaccines needed primarily by developing countries and travelers, including vaccines against bacterial enteric infections, such as ETEC. The CVD's Clinical Research Unit has conducted many clinical trials, including multiple trials of ETEC vaccine candidates including, LT, mLT, and dmLT. The Unit is supported by the CVD's Regulatory Affairs & Quality Management Office, with both domestic and international regulatory affairs specialists, and a quality management coordinator. CVD Research Labs are fully equipped for immunology, molecular biology, and bacteriology, including safe handling of vaccines and bacteria.

The CVD Immunology Group is composed of 3 integrated components that work together using state-of-the-art equipment to measure a broad array of immunologic responses: a Cellular Immunology Section, a Flow Cytometry Core, and an Applied Immunology Unit.

The CVD's Immunology Group performs the protocol-required assays for DMID 09-0066 (oral dmLT), DMID 12-0023 (sublingual dmLT), and DMID 13-0013 (intradermal dmLT). Thus, training and sharing of the methods and reagents by the staff of the Immunology Group will be central to ensuring that the assays at icddr,b are qualified for the present study.

The CVD's Flow Cytometry Core houses: a Beckman-Coulter (former Dako-Cytomation) MoFlo flow cytometer/cell sorter system equipped with 3 lasers (i.e., up to 12 simultaneous parameters), a custom Becton-Dickinson LSR-II Flow cytometer analyzer equipped with 4 lasers (i.e., up to 16 simultaneous parameters), and a DVS Sciences CyTOF Mass Cytometer, which uses mass spectrometry to allow the simultaneous detection of at least 35 metal-labeled antibodies. The CyTOF Mass Cytometer will be used for the T cell assays in the present study.

International Centre for Diarrhoeal Disease Research, Bangladesh (icddr,b) - Dhaka, Bangladesh

The icddr,b is a renowned international research institute addressing fundamental global health needs; a leader in endemic diarrheal disease research, including ETEC. For the present study, the icddr,b will operate under a subcontract with the CVD. The icddr,b routinely is engaged in research, training, and extension activities and also provides significant clinical services to thousands of impoverished patients. The centre has both national and international staff, including public-health scientists, epidemiologists, demographers, social and behavioral scientists, laboratory scientists, clinicians, nutritionists, Information Technology (IT) professionals, and research experts in emerging and re-emerging infectious diseases and vaccine sciences.

The icddr,b main campus is located in Dhaka (the main hospital, clinical labs and all extensive research laboratories are at this site). The centre also has a variety of urban and rural field sites, all of which have been extensively involved in vaccine research, clinical trials and observational studies. There are three major field sites available: Matlab, Mirpur, and Bandarban.

Mirpur Field Site: The Mirpur research site is located in the Dhaka Metropolitan area, approximately 7 km from the main icddr,b Dhaka hospital, and has been used for field studies since 1987. Mirpur is densely populated with approximately 3.5 million individuals. This research clinic has enjoyed a long-standing relationship with this stable community. The Mirpur research clinic is composed of five floors, with each floor consisting of 4 rooms; approximately 7000 square feet of research space. This dedicated clinic building is situated within the same neighborhood as the study population. The facility contains a participant waiting area, several examination rooms, a staff work and file room, a specimen processing area, archive room and a meeting room. The specimen processing area contains a refrigerator and a centrifuge with 24 hour generator back-up. The building has 24 hours security coverage and internet service. All clinical and laboratory specimens are sent to the clinical and research laboratories of the main icddr,b campus daily maintaining cold chain requirements. Numerous natural history studies as well as GCP based vaccine trials have been conducted or planned in this site and include oral cholera, oral ETEC vaccine(s) and typhoid vaccine studies. Socio-demographic information is also available from this area. A geographic information system (GIS) was used for mapping of this area and identifying households and other information of these households have been completed using PDA (Personal digital assistance) devices and android TABs.

The Mucosal Immunology & Vaccinology Laboratory at the icddr,b is a BSL2 level facility with internal and external quality assurance procedures that are carried out for the laboratory assays and their results. All standard operating procedures (SOPs) for studies are updated frequently for meeting study requirements. The laboratory is equipped with six biohazard safety hoods for

processing of biological samples and for maintaining sterile conditions for specimen processing. For the fractionation of samples, refrigerated table top centrifuges, high speed centrifuges (Beckman) and ultracentrifuges (Beckman L7-80 and a Beckman L5-65B) are also available. Incubators with carbon-dioxide gas attachment are present for the study of B cell and T cell responses. There is a cryostat for sectioning of frozen sections and a microtome for paraffin sections. Four ELISA readers including two kinetic ones are linked to a computer for determining antibody responses in study samples. Facilities for ELISPOT and "Antibody in Lymphocyte Supernatant" (ALS) assays are available. A cytotoxic T cell (CTL) automatic counter and stereomicroscopes, for enumeration of ASCs, are available. There are facilities for carrying out extraction of lymphocytes from gut biopsies. In addition, facilities for carrying out intestinal lavages and fecal extracts are used for assessing mucosal antibody responses. There are six -80°C low temperature freezers, two liquid nitrogen freezers, and a number of additional freezers (-20°C) and refrigerators used for storage of specimens from vaccine trial and studies of infectious diseases. The Flow Cytometry Core facility that is coordinated by and used by this lab have a Becton Dickinson Facs Caliburs and FACSAria™ III cell sorter. The FACSAria™ III is currently configured with 3 lasers and is capable of detecting 9 fluorochromes (this capacity is expandable if needed) as well as a 96-well plate adapter for single cell sorting.

2.1.2 Clinical Experience with Oral dmLT

The National Institute of Allergy and Infectious Disease (NIAID) sponsored a Phase 1 dose-escalation study conducted at the CVD and University of Cincinnati in 36 healthy adult volunteers who received a single oral dose of 5, 25, 50, or 100 µg dmLT (DMID Protocol 09-0066, under IND 14399; NCT01147445). The immune responses were measured by dmLT-specific serum IgA and IgG, fecal IgA, ASC, and ALS assays. No participants experienced diarrhea in any dosage group and the vaccine was well tolerated. Although the immune responses were limited in those receiving 5 µg or 25 µg of dmLT, the immune response was greater in those receiving 50 µg or 100 µg of dmLT, with a possible plateauing of immune responses at 50 µg[34].

A Phase 1 study of an inactivated oral multivalent ETEC vaccine (ETVAX, expressing CFA/I, CS3, CS5, CS6, and LTB) with dmLT as an adjuvant was conducted in 129 healthy Swedish adults. The vaccine with dmLT was safe and well tolerated; dmLT appeared to further enhance mucosal immune responses to CF antigens which were present in low amounts in the vaccine.[35] A Phase 1/2 study of oral administration of an inactivated ETEC vaccine (ETVAX) with and without dmLT as an adjuvant was initiated October 2015 and is ongoing at the icddr,b (VAC 014/OEV-122; NCT02531802).

A Phase 2 study of the oral ETEC vaccine (ETVAX) administered with dmLT is currently being conducted among healthy adults in Bangladesh by icddr,b. (NCT02531802) investigators.

A Phase 1/2b study of a live, attenuated oral ETEC vaccine (ACE527) adjuvanted with dmLT was conducted in the U.S. (VAC 006; NCT01739231). The trial results have not yet been published, but the data have been presented at scientific conferences. Immunization with ACE527 administered with or without dmLT was safe and immunogenic. The addition of dmLT to the vaccine formulation did not significantly change its safety or immunogenicity profile. When a total of 57 participants were challenged with ~10^7 cfu ETEC strain H10407, participants in the ACE527+dmLT vaccine group were highly protected; only 3/13 subjects developing severe diarrhea (PE=65.9%, P=0.003 one-sided Barnard's test). Meanwhile, 7/13 (53.8%) subjects receiving ACE527 alone developed severe diarrhea (PE=20.5%; P=0.205). ACE527+dmLT was also 58.5% efficacious in protecting against diarrhea of any severity (P=0.016) and substantially reduced H10407 shedding post-challenge. There were no significant adverse events (AEs) associated with orally administered dmLT as adjuvant.[36]

2.1.3 Clinical Experience with Sublingual dmLT

NIAID is currently sponsoring a Phase 1 dose-escalation study in 64 healthy adult volunteers who are to receive three sequential SL doses of 1, 5, 25, or 50 µg dmLT; a final cohort will compare sublingual vs. oral administration at the dose level showing optimal immunogenicity without any safety concerns (DMID Protocol 12-0023, under IND 14399; NCT02052934). Delivery of dmLT to Cohorts 1-4 were completed and no safety concerns were identified with up to the 3 doses of 50 µg dmLT delivered orally. The CVD's Applied Immunology Laboratory has performed all the immunologic assays for this study. The study is completed; there have been no noted safety concerns, currently a clinical study report (CSR) is being finalized . There are no other human trials, to our knowledge, that have been completed using SL route of administration of dmLT as a vaccine or adjuvant.

2.1.4 Clinical Experience with Intradermal dmLT

NIAID is sponsoring a Phase 1 dose-escalation study of three sequential ID doses of 0.1, 0.3, 1.0, or 2.0 µg dmLT, spaced by 21 days (DMID Protocol 13-0013, under IND 16836 NCT02531685). The CVD's Applied Immunology Laboratory is performing all the immunologic assays for this study. DMID 13-0013 trial started in April of 2016 at the CCHMC, and it is the first in human trial with dmLT administered via the ID route. Currently, three doses of 0.1 µg, 0.3 µg and1.0 µg have been delivered to subjects; no safety signals were seen nor any halting rules were met.
The first generation mLT (LTR192G), which is more toxic in animal (IB V1.0 16 May 2017) than the double-mutant being tested here, was evaluated in a phase 1 trial of ID administered CfaE with or without 100 ng LTR192G. In this study, local reaction at the vaccine injection site were the most common vaccine-related adverse events and were generally mild in severity.

Hypo/hyperpigmentation were observed for 6 months in 45% of the participants and up to 1 year in 18% of the study participants. Preliminary immunogenicity results have revealed robust anti-LTB and anti-CfaE serologic and ASC responses (IgG/IgA), as well as high hemagglutination inhibition titers after ID immunization[37].

2.1.5 Rationale

ETEC remains a major cause of childhood diarrhea in low-middle income (i.e., developing) countries and is endemic to areas with poor infrastructure for sanitation and without access to clean water. The use of dmLT is proposed as an oral, sublingual, or intradermal traveler's diarrhea (TD) vaccine for persons from industrialized (i.e., developed) countries that visit high-risk developing (non-industrialized) countries. It is envisioned that dmLT may also be used as a component of candidate ETEC vaccines under development for use in infants and young children living in ETEC endemic areas. The vaccine should provide its best protection against ETEC diarrhea associated with infection by strains expressing the LT toxin alone or in combination with ST toxin (LT/ST). Native LT given by the transcutaneous immunization (TCI) route has been shown to protect travelers against TD due to LT producing ETEC strains in two field trials; [8,15] however, broader protection against ETEC might be achievable if the vaccine is given by the mucosal route (oral or SL), or by routes that may induce both systemic and mucosal immunity such as ID. Non-native LT vaccine such as dmLT may have beneficial effects beyond the anticipated protection against ETEC expressing LT or LT/ST. Two cellular ETEC vaccines that have recently completed clinical trials included study arms in which dmLT was added as a vaccine component to improve the anti-LT toxin responses induced by the vaccine, as well as potentially improve mucosal responses to other co-administered ETEC surface antigens. In the case of ETVAX vaccine, the anti-LT toxin and anti ETEC colonization factor antigen immune responses were significantly improved with the addition of the lower dose of dmLT adjuvant.[26] Furthermore, adding dmLT to the live attenuated ETEC vaccine formulation offered significant protection against ETEC challenge 6-7 months after the primary immunization series that was otherwise not conferred by vaccine alone.[38]

Compared to oral immunization, SL or ID immunization may increase dose-sparing and potentially eliminate the need for buffering to neutralize gastric acid, therefore providing greater protein antigen stability. Both the SL and ID routes may also help bypass gut enteropathy, which serves as a major barrier to oral vaccination. The increased dose sparing would help reduce the cost of the final vaccine product.

The optimal protection to be afforded against enteric diarrheal pathogens is believed to be through the elicitation of robust mucosal immune responses. The addition of LT may serve as both a protective antigen and/or a potent immunostimulating adjuvant to vaccine candidates.

The aforementioned DMID-sponsored studies on dmLT have been or are being conducted in the U.S. (an industrialized country) and there are unanswered questions regarding the immune responses through different routes of administration in a non-industrialized country. This study is designed to assess the safety and immune responses of a range of doses of dmLT when administered by the oral, SL, or ID route, in volunteers residing in an endemic country for ETEC disease. The proposed indication for dmLT is to protect against ETEC diarrhea associated with infection by strains expressing the LT toxin alone or in combination with the heat-stable toxin (ST) as a component of an oral, sublingual, or intradermal diarrhea vaccine for those residing in high risk endemic regions as well as international travelers to these high-risk regions.

2.2 Potential Risks and Benefits

2.2.1 Potential Risks

The study product, dmLT, is a non-infectious product; the risk of direct transmission from study participants to the community or research members is negligible. The study product has been given to humans as a stand-alone single component antigen and as an adjuvant with other candidate vaccines. There have been few adverse events reported. Any candidate vaccine has the risk of allergic reaction; these reactions are rare and unpredictable.

Risk of AEs when dmLT is administered by the oral route. The enterotoxicity observed with native LT has, in theory, been eliminated with the two mutations that have been introduced. There is the possibility of diarrhea with dmLT, if there is residual enterotoxicity. Other possible adverse effects of residual enterotoxicity might include abdominal cramping or discomfort, gas or bloating, nausea, vomiting and/or decreased appetite.

Risk of AEs when dmLT is administered by the SL route. Sublingual administration of dmLT is not expected to allow for direct contact with the nasal passages and thus there is no expectation for Bell's palsy. Whereas, intranasal delivery of native LT (and a K63 mutant) with influenza and other experimental vaccines has been shown to be associated with transient peripheral facial nerve palsies (Bell's palsy) in a small but significant number of volunteers.[33,39] These events were attributed to retrograde axonal transport of the toxin by binding neuronal ganglioside and/or an inflammatory immune response.[40,41] Other possible adverse events are irritation of the tongue or oral cavity. As with oral dmLT, there may be a risk for mild enterotoxicity, including abdominal cramping or discomfort, gas or bloating, nausea, vomiting and/or decreased appetite.

Risk of AEs when dmLT is administered by the ID route. dmLT administered via the ID route is currently being tested in humans under the DMID 13-0013 protocol. ID administration of 100 ng or 300 ng of dmLT to two separate cohorts of the 10 subjects each is completed. To

date no safety concerns were noted, and a 3rd cohort to receive 1 μg is planned to start in July 2017. Nonetheless, ID vaccination may be associated with injection site edema, erythema, pain, pruritus, induration, plaques, hypopigmentation, hyperpigmentation, and vesicles in a dose-dependent manner.

Risk with venipuncture and blood collection. Drawing blood may cause transient discomfort and fainting. Fainting is usually transient and managed by having the participant lie down. Bruising at the blood draw site may occur, but can be prevented or lessened by applying pressure to the venipuncture site for several minutes. Breaking the skin during venipuncture or ID vaccination may cause transient discomfort, fainting, or infection. The use of sterile technique and materials will minimize the risk of infection.

Risk to breach of confidentiality. Participants will be asked to provide personal health information (PHI). All attempts will be made to keep this PHI confidential, within the limits of the local laws and regulations. There is the chance that unauthorized persons will gain access to PHI. To minimize this risk, all records will be maintained in locked file cabinets or locked rooms, when not in use. When in use, research records will be under the direct supervision of study team members. Electronic files will be password protected. Only persons who are involved in the conduct, oversight, monitoring, or auditing of this study will be allowed access to the PHI that is collected.

There may be other unknown risks, discomforts, or side effects from dmLT. Any significant adverse events which are deemed possibly related to dmLT will be disclosed, as appropriate.

2.2.2 Known Potential Benefits

The receipt of dmLT could provide partial protection against an infection with ETEC. Because this study is placebo-controlled and allocation is random and double-blinded, all participants should assume no definitive direct benefit. Nonetheless, due to the global public health importance of ETEC infections and vaccines which could be adjuvanted with dmLT, participants provide a greater societal benefit for helping the study team members gain important knowledge on dmLT.

2.2.3 Risk Benefit Ratio

The burden of ETEC infections in Bangladesh is high, it is a country with endemic ETEC, where ETEC infections are common. There is the possibility of some protection with participation in this study, but some participants will receive placebo and the vaccine may not work. Previous studies of the vaccine have not demonstrated excessive toxicity or reactogenicity. The assessment of the safety and tolerability of the vaccine is the primary objective. There is also a potential for a societal benefit, in that an ETEC vaccine may be helpful for decreasing the burden

associated with this common infection. Therefore, the risks of the study are balanced by benefits.

3 OBJECTIVES

3.1 Study Objectives

Primary:

- To assess the reactogenicity, safety and tolerability of dmLT when administered in three sequential doses, over a range of dosages by oral, sublingual, or intradermal routes

Secondary:

- To assess the long-term safety, from first vaccination through 6 months following the last dose of vaccine
- To evaluate the serum anti-dmLT IgG and IgA response
- To evaluate the IgG and IgA anti-dmLT Antibody Secreting Cell (ASC) response
- To evaluate the IgG and IgA anti-dmLT Antibodies in Lymphocyte Supernatant (ALS) response
- To evaluate the total fecal IgA and fecal anti-dmLT IgA response
- To evaluate the total salivary IgA and the saliva-derived anti-dmLT IgA response

Exploratory:

- To measure the mucosal homing of IgA anti-dmLT ASC
- To measure the serum toxin neutralizing antibody response
- To measure the dmLT-specific IgG and IgA memory B cell response
- To determine the dmLT-specific effector and memory T cell responses

3.2 Study Outcome Measures

3.2.1 Primary Outcome Measures

The **Primary Safety Endpoints** to be used to evaluate reactogenicity, safety and tolerability are as follows:

- The occurrence of solicited local site and systemic reactogenicity events from vaccination through 7 days after each dose of vaccine is administered
- The occurrence of study withdrawals throughout the study
- The occurrence of discontinuation of study vaccination during the study

- The occurrence of unsolicited vaccine-related adverse events (AE), including laboratory AE, from first vaccination through 28 days after the last dose of vaccine is administered

3.2.2 Secondary Outcome Measures

The **Secondary Safety Endpoints** to be used to evaluate the safety of this study are:

- The occurrence of vaccine-related serious adverse events (SAE) from the first vaccination through 6 months after the last dose of vaccine is administered

The **Secondary Immunogenicity Endpoints** to be used to evaluate the immune response to vaccination will consist of the following:

- At any time after vaccination, the proportion of participants with a ≥4-fold rise in dmLT-specific serum IgG and IgA titers over baseline measured by ELISA
- At any time after vaccination the proportion of participants with >8 dmLT-specific IgA or IgG ASC / 10^6 PBMC as measured by ELISpot
- At any time after vaccination, the proportion of participants with ≥2-fold rise in ALS anti-dmLT-specific IgG and IgA titers over baseline measured by ELISA
- At any time after vaccination, the proportion of participants with a ≥4-fold rise over baseline in dmLT-specific fecal IgA titers measured by ELISA
- At any time after vaccination, the proportion of participants with a ≥4-fold rise over baseline in dmLT-specific salivary IgA titers measured by ELISA

3.2.3 Exploratory Outcome Measures

The **Exploratory Endpoints** to be used to evaluate the immune response to vaccination will consist of the following:

- Geometric mean titers of dmLT-specific IgG and IgA in ALS measured by ELISA at time points listed in Appendix A
- Geometric mean titers of dmLT-specific serum IgG and IgA measured by ELISA at time points listed in Appendix A
- Mean and median number of dmLT-specific IgG and IgA ASC measured by EliSpot at time points listed in Appendix A
- Geometric mean titers of dmLT-specific fecal IgA measured by ELISA at time points listed in Appendix A
- Geometric mean titers of dmLT-specific salivary IgA measured by ELISA at time points listed in Appendix A

- The proportion of participants with dmLT-specific memory B cell response as measured by ELISpot at time points listed in Appendix A
- At any time after vaccination, the proportion of participants with anti-dmLT IgG and IgA ASC in circulation expressing gut homing receptors (integrin α4β7 in the absence or presence of CD62L) measured by EliSpot assay at time points listed in appendix A
- At any time after vaccination, proportion of participants with ≥4-fold rise over baseline in toxin neutralization titers measured by Y-1 cell assay
- At any time after vaccination, the proportion of participants with dmLT-specific effector and memory T cell responses as measured by CyTOF at time points listed in appendix A

4 STUDY DESIGN

This is an outpatient Phase 1 double-blinded, placebo-controlled, dose-escalation trial in approximately 135 (plus any additional participants if needed to have minimal evaluable participants / cohort as described in Table 1) apparently healthy adult volunteers, age 18-45 years, who meet all the eligibility criteria and reside in Bangladesh, an endemic country for ETEC infection. The study population will be recruited from Mirpur, a densely populated urban slum community of Dhaka, Bangladesh, known as having a high rate of diarrheal, respiratory and enteric disease.

This clinical trial is designed to assess the safety, reactogenicity, tolerability, and immunogenicity of a range of dosages of dmLT administered by three different routes: oral, SL, or ID. The schematic of study design is presented in Table 1. The oral and SL routes of administration will evaluate dosages of 5, 25, and 50 µg of dmLT; the ID route of administration will evaluate dosages of 0.3, 1.0, and 2.0 µg of dmLT. Participants will receive a total of three sequential doses of dmLT; the oral and SL routes will be at days 1, 15, and 29 and the ID route will be at days 1, 22, and 43. After each dose of study product, participants will be observed for 30-90 minutes, depending on the route of administration.

Safety, reactogenicity, and tolerability of the study products will be evaluated for each participant. Solicited local and systemic reactogenicity will be collected for 7 days after each vaccine administration. Memory Aids and digital thermometers will be given to participants to record solicited local and systemic reactogenicity including elevated body temperature (i.e., fever) at home, and they will be reviewed with the participants. Unsolicited AEs will be collected from the time of each study vaccination through 28 days after the last vaccination. Safety laboratories will be collected on Visit 7 or about 28 days after the last vaccination. Serious adverse events (SAEs) will be collected from the time of the first study vaccination through 6 months after the last study vaccination. Tolerability of the study products will be assessed by recording the occurrence of and reason for study withdrawals and discontinuation of study vaccination during the study.

The evaluation of immunogenicity will include dmLT-specific antibody responses in serum, fecal, salivary and ALS samples, in addition levels of ASC in circulation will be determined. Memory B cells specific for dmLT will also be evaluated. An in-depth evaluation of effector and memory T cells response, including homing potential, cytokine/chemokine production profile, degranulation capacity, and activation potential, will also be performed. In order to maximize our ability to compare immune responses between US and Bangladesh studies, the timing of the sample collections for the immunological assays needs to be comparable. The timing of the specimen collection for the immunological assays were selected based on previous

DMID-funded dmLT studies conducted in the U.S. (i.e., DMID protocols 09-0066 and 12-0023) and the ongoing study 13-0013.

Additional participants may be enrolled to ensure there are at least 11 evaluable participants (10 vaccinees and 1 placebo) in each of the cohorts (cohorts A through I). Evaluable participants are defined as completing all 3 doses of vaccine and providing both safety and protocol-required specimens through at least 7 days after the third dose of vaccine. If additional participants are required, to maintain blinding, treatment assignments of additional participants are to be determined by Emmes.

The duration of the study for each participant will be up to 9 months (inclusive of the screening period through 6 months after the third dose of vaccine, plus a 2-week window on the last visit), depending on the route of dmLT administered. Details on the study procedures, evaluations, and study schedule are in Sections 7, 8 and Appendix A, respectively.

4.1 Dose Escalation Halting Criteria

Prior to dose escalation to the next cohort within a route of administration group (see Table 1), safety data through 7 days post vaccination of the third dose will be reviewed. Specifically, the Statistical and Data Coordinating Center (SDCC) will provide the cumulative safety data and notify the protocol PI, medical monitor (MM), and DMID Independent Safety Monitor (ISM), that the following criteria have not been met based on a review of the safety data collected in the seven days after the third vaccination of the last participant in the current cohort. Only one cohort will be enrolled at a time, and enrollment will proceed from Cohort A to B; Cohort C to D, and Cohort E to F if the halting criteria described in Section 9.5 are NOT met.

In addition, there will be no progression to the next cohort within a route of administration group if the following dose escalation halting criteria is met:

- The site PI, protocol PI, MM, DMID ISM or local DSMB identify a safety concern that would prompt a review by the DMID DSMB.

If the above dose escalation halting criteria are NOT met, the dose escalation may proceed to the next successive route of administration cohort (see section 9.6.3).

If any of the above dose escalation halting criteria are met, then escalation to the next successive route of administration cohort will not proceed and the data will be reviewed by the DMID DSMB (see section 9.6.1). The enrollment for the next cohort may begin when all three of the below requirements are met:

- DMID DSMB recommendation to proceed,
- DMID concurs with the DMID DSMB recommendation to proceed,

- Local DSMB recommendation to proceed.

Upon completion of Cohorts A through F, a scheduled DMID DSMB meeting will be convened to review all the safety data (clinical labs and reported events) collected in the seven days after the third vaccination in each cohort. Progression to Cohorts G, H and I will occur when all three of the below requirements are met:

- DMID DSMB recommendation to proceed
- DMID concurs with the DMID DSMB recommendation to proceed
- Local DSMB recommendation to proceed

As the IND sponsor, DMID retains the final decision-making authority to stop the study at any point.

5 STUDY ENROLLMENT AND WITHDRAWAL

5.1 Subject Inclusion Criteria

Study participants are eligible for this study if they fulfill the inclusion criteria below:

1. Male or female age 18-45 years old, inclusive.
2. Provides written informed consent before initiation of any study procedures.
3. Healthy as judged by the site investigators and determined by medical history, medication history, and physical examination.
4. Capable of understanding, consenting, and complying with all the study visits and procedures.
5. Body Mass Index of no less than 18.5
6. Agrees not to participate in another clinical trial during the study period.
7. Agrees to complete all study visits and procedures.
8. Agrees not to donate blood to a blood bank for 12 months after receiving the last vaccine.

5.2 Subject Exclusion Criteria

Participants will be ineligible for any of the following conditions or reasons:

1. Women who are pregnant or lactating or have a positive urine pregnancy test at screening or on the day of vaccinations.

   Note: all women presenting for screening will have urine pregnancy testing. "Females of childbearing potential must agree to use an efficacious hormonal or barrier method of birth control from screening and through 28 days post last dose of vaccine. Abstinence is also acceptable."

2. Presence or history of a chronic medical condition* that would, in the opinion of the investigator, render vaccination unsafe or interfere with the evaluation of the vaccine.

   ** Note: this may include, but is not limited to: significant renal disease, unstable or progressive neurological disorders, diabetes, heart disease, asthma, lung disease, liver disease, organ transplant recipients and cancer.*

3. Presence of a significant dermatologic condition*, or tattoo(s), scarring or significant skin damage at the vaccination site that would impede evaluation of local reactogenicity.

   ** Note: this may include severe eczema, psoriasis or history of keloid formation. Participants with history of squamous cell or basal cell skin cancer that has been surgically excised and*

considered cured may be enrolled in the study if the skin cancer site is healed and is not at proposed vaccine administration site.

4. Any developmental abnormality of the palate.
5. Participants diagnosed with autoimmune disorders, chronic inflammatory disorders or neurological disorders with a potential autoimmune correlation.
6. Use of long-term (≥2 weeks) oral steroids, intranasal or topical prednisone (or equivalent), parenteral steroids, or high-dose inhaled steroids (>800 μg/day of beclomethasone dipropionate or equivalent) within the preceding 6 months.
7. Has major psychiatric illness* during last 12 months that in the investigator's opinion would preclude participation.

   ** Note: Participants taking antipsychotic or antimanic drugs should not be enrolled. These include: aripiprazole, clozapine, ziprasidone, haloperidol, molindone, lamotrigine, gabapentin, topiramate, loxapine, thioridazine, thiothixene, pimozide, fluphenazine, risperidone, mesoridazine, quetiapine, trifluoperazine, chlorprothixene, chlorpromazine, perphenazine, olanzapine, carbamazepine, divalproex sodium, lithium carbonate, or lithium citrate. Participants taking a single antidepressant drug and are stable without de-compensating symptoms in the preceding 3 months can be enrolled in the study.*

8. Use of prescription or over-the-counter (OTC) anti-inflammatory medications* 48 hours prior to receiving the investigational product.

   ** Note: This includes naproxen, aspirin, ibuprofen, and other non-steroidal anti-inflammatory drugs.*

9. Gastrointestinal symptoms* in the past 24 hours or abdominal pain lasting for more than 2 weeks in the past 6 months.

   **Note: this may include, but is not limited to: abdominal pain or cramps, loss of appetite, nausea, general ill-feeling or vomiting.*

10. Moderate or severe diarrheal illness* during the 6 weeks prior to enrollment.

    **Note: Moderate or severe diarrheal illness is defined by the passage of ≥ 4 unformed or loose stools (mix of liquid and solid components) in a 24 hour period*

11. History of chronic gastrointestinal illness*.

    **Note: this includes severe dyspepsia or gastroesophageal reflux disease, constipation, irritable bowel syndrome (IBS), hemorrhoids, diverticular disease, colitis, colon polyps, colon cancer, and inflammatory bowel disease. Mild or moderate heartburn or epigastric pain occurring no more than three times per week is permitted.*

12. Regular use (weekly or more often) of laxatives, anti-diarrheal, anti-constipation, or antacid therapy.

13. History of major gastrointestinal surgery, excluding uncomplicated appendectomy or cholecystectomy.

14. History of systemic antimicrobial treatment (i.e., topical treatments are not an exclusion) during the week prior to any administration of dmLT.

15. Acute febrile illness (body temperature ≥38°C) during the week prior to enrollment.

16. Abnormal screening laboratories*.

    **Note: screening labs include white blood cell count (WBC), absolute neutrophil count (ANC), hemoglobin (Hg), platelet count, serum creatinine, serum albumin, alanine aminotransferase (ALT, also known as SGPT), and serologic testing for Hepatitis B virus surface antigen (HBsAg) and Hepatitis C virus (HCV) antibody. See Appendix B for acceptable values.* Abnormal vital signs. See *Appendix C* for abnormal values.

17. Isolation of specific bacteria* from screening stool cultures.

    **Note: bacteria include ETEC, Vibrio cholerae, and Shigella spp. Salmonella and Campylobacter will not be evaluated as part this criterion.*

18. Received an inactivated licensed vaccine within 2 weeks of enrollment or live licensed vaccine within 4 weeks of enrollment.

19. Received a cholera (licensed or experimental) vaccine, *E. coli* vaccine, or *Shigella* vaccine in the last 3 years.

20. History of receiving immune globulin or other blood product within the 3 months before enrollment in this study.

21. Currently enrolled in another study, involving an experimental agent. Participants involved in observational studies or surveys remain eligible.

22. Any condition that would, in the opinion of the Site Investigator, place the participant at an unacceptable risk of injury or render the participant unable to meet the requirements of the protocol.

23. Known allergies to study compound or components of the study vaccine.

24. Donating blood in the 8 weeks prior to study entry.

5.3 Treatment Assignment Procedures

5.3.1 Randomization Procedures

No exemptions are granted on Subject Inclusion/Exclusion Criteria in DMID-sponsored studies. Questions about eligibility should be directed toward the DMID Medical Officer. Per International Conference on Harmonisation (ICH) guideline E6: Good Clinical Practice (GCP), screening records will be maintained to document the reason why an individual was screened, but failed trial entry criteria. Screening information will be recorded in the Statistical and Data Coordinating Center's (SDCC's) Advantage eClinical[SM] (Electronic Data Capture System).

Once consented and upon entry of demographic data and confirmation of eligibility for this trial, the participant will be enrolled. Enrollment of participants will be done online, using the enrollment module of Advantage eClinical[SM]. Participants within each cohort will be randomly assigned to receive study product or placebo, as shown in Table 1. The list of randomized treatment assignments will be prepared by statisticians at the SDCC and included in the enrollment module for this trial. Advantage eClinical[SM] will assign each participant a treatment code from the list after the demographic and eligibility data have been entered into the system. A designated individual at the site will be provided with a treatment key, which links the treatment code to the actual treatment assignment, which will be maintained in a secure location.

Instructions for use of the enrollment module are included in the Advantage User's Guide. Manual back-up randomization procedures and instructions are provided in the MOP for use if the site temporarily loses access to the Internet or the online enrollment system is unavailable.

The enrollment and randomization for all cohorts will be done as a dose-escalation, with the lower dosage cohort enrolled before the higher dosage cohort within a given route of administration (Table 1).

5.3.2 Masking Procedures

This is a double-blind study. While participants will not be blinded to cohort, they will be blinded to group assignment (i.e., placebo vs. vaccine) within a cohort.

Participants, investigators, and study personnel performing any study-related assessments following study product administration, and laboratory personnel performing immunologic assessments will be blinded to the administration of vaccine or placebo, within each cohort.

The randomization scheme will be generated by the SDCC and provided to the assigned unblinded study personnel (e.g., pharmacist preparing study products and unblinded study product administrators).

The unblinded study product administrator is a study personnel licensed, registered, or certified to administer vaccines, but will not be involved in study-related assessments or have participant contact for data collection following study injection.

Both DSMBs may receive data in aggregate and blinded by treatment arm, or may be unblinded to individual participant treatment assignments, as needed, to adequately assess safety issues. There will be one designated physician within the Independent Protocol Safety Team (IPST) that will be assigned to communicate with CROMS Pharmacovigilance for purposes of reporting SAEs and to request unblinding (see Section 9.6.4 and MOP for additional details).

5.3.3 Reasons for Withdrawal and Discontinuation of Dosing

A participant may voluntarily withdraw their consent for study participation at any time and for any reason, without penalty.

A participant may be withdrawn from study participation for any of the following reasons:

- Medical disease or condition, or any new clinical findings for which continued participation, in the opinion of the investigator, would compromise the safety or welfare of the volunteer, or would interfere with the volunteer's successful completion of the study, or would interfere with the evaluation of responses.
- Participant no longer meets eligibility criteria.
- As deemed necessary by the investigator for noncompliance or other reasons.
- Participant withdrawal of consent.
- Participant lost to follow-up.
- Participant dies.
- Termination of this study.
- New information becomes available that makes further participation unsafe.

The second or third dose of study product will not be administered for any of the following reasons:

- Medical condition for which continued participation, in the opinion of the investigator, would pose a risk to the participant or would likely confound interpretation of the results.
- Presence of signs or symptoms that could confound or confuse the assessment of reactogenicity. Study vaccination should be postponed/deferred until signs, symptoms, or acute illness have resolved and if within acceptable protocol-specified window for that visit. If outside this window, the DMID Medical Officer must first approve the vaccination and the documentation of approval should be filed in the research record.
- Any unresolved or continuing solicited or unsolicited Grade 3 AE deemed to be vaccine related until the time of next dose. An unresolved or continuing Grade 1 or 2 AE is

permissible unless, in the opinion of the investigator, it would render study vaccination unsafe or interfere with the evaluation of responses.

- Solicited or unsolicited Grade 3 AE that occurs without alternative etiology through 7 days following the previous vaccinations.
- Solicited AEs, the reactogenicity events occurring through 7 days of each vaccine:
    - Systemic reactions to include: feverishness (chills/shivering/sweating), fatigue (tiredness, malaise, general unwell feeling), myalgia (body ache, muscular pain) headache, diarrhea*, nausea*, vomiting* or abdominal discomfort*. Systemic reactions will also include the measurement of a daily oral temperature.
    - Local reactions include:
        - For oral: irritation of oral cavity or tongue, diarrhea, nausea, vomiting, abdominal discomfort
        - For SL: irritation of oral cavity or tongue, or facial nerve disturbance, diarrhea, nausea, vomiting, abdominal discomfort
        - For ID: injection site pain, redness, swelling, bruising, itching, hypo/hyper pigmentation and induration, vesicles or hardened mass

    * For the oral and SL routes of administration, diarrhea, nausea, vomiting, and abdominal discomfort will be evaluated as local reactions.
- Participant no longer meets eligibility criteria (e.g., new onset illness or condition that meets exclusion criteria)
- As deemed necessary by the investigator for noncompliance or other reasons.
- Participant refusal of further study vaccinations.
- Participant withdrawal of consent.
- Participant lost to follow-up.
- Termination of this study.
- New information becomes available that makes further participation unsafe.

5.3.4 Handling of Withdrawals

The primary reason for withdrawal from this study will be recorded on the Study Status data collection form. Participants will be encouraged to complete the Early Termination Visit. The Early Termination Visit procedures are listed in Section 7.5.

The investigator will make at least three documented attempts to contact any participant who does not return for scheduled follow-up. Although the participant is not obliged to give reasons for withdrawing early, the investigator should make a reasonable effort to ascertain the reason(s) while fully respecting the participant's rights. An investigator may also withdraw a participant from the study. If a participant withdraws early, an early withdrawal assessment should be

obtained via a clinic visit or via telephone contact if this is the only available means. Attempts will be made to follow all AEs through resolution, as applicable. Any withdrawals, terminations, or losses to follow up will be recorded on the appropriate source document.

Participants who have received the first vaccination may choose to discontinue receipt of study vaccine for any reason but may choose to remain in the study (i.e., not withdraw from study). In addition, a participant may be discontinued from receipt of the second or third vaccination. However, a discontinuation from vaccination will not result in automatic withdrawal from the study. Participants meeting criteria for discontinuation of study vaccination must not receive any further study vaccine, but should continue to be monitored for safety and immunogenicity if this does not result in a safety risk for the participant and the participant agrees to continue with safety and immunogenicity monitoring. Participants that voluntarily discontinue additional doses of vaccine should also be monitored for safety and immunogenicity, if the participant consents.

Participants who provide informed consent and are randomized but do not receive study product may be replaced.

For the oral cohorts, participants that vomit or spit out the first dose of study product within 90 minutes of taking the study product will be replaced. Any participant with a failure in administering or receiving a first dose of study product will be replaced.

Participants who withdraw, or are withdrawn or terminated from the study, or are lost to follow-up after signing the informed consent form, randomization, and receipt of study vaccine will not be replaced.

Additional participants may be enrolled to ensure there are at least 11 evaluable participants in each cohort (i.e., 10 vaccinees and 1 placebo per cohort). Evaluable participants are defined as at least completing 3 doses of vaccine and providing both safety and protocol-required specimens through at least 7 days after the third dose of vaccine.

If replacement or additional participants are required and because the study will remain double-blinded, these participants are to be determined by Emmes. The procedures for replacement of study participants can be found in the *Manual of Procedures* (MOP). Any withdrawals, terminations, or losses to follow up will be recorded on the appropriate source document.

5.3.5 Termination of Study

Although the study Sponsor has every intention of completing this study, the Sponsor reserves the right to terminate the study at any time for clinical or administrative reasons. Reasons for

termination include, but are not limited to study closure due to DSMB review and recommendation and at the discretion of DMID (Sponsor).

6 STUDY INTERVENTION/INVESTIGATIONAL PRODUCT

6.1 Study Product Description

LT(R192G/L211A), or dmLT, is a derivative of wild-type ETEC heat-labile enterotoxin that has been genetically modified by replacing the arginine at amino acid position 192 with glycine and the leucine at amino acid position 211 with alanine. These two amino acid substitutions take place in proteolytic cleavage sites which are critical for activation of the secreted toxin molecules. The protein has been designated LT(R192G/L211A) and has been extensively evaluated in pre-clinical animal studies for its ability to induce anti-dmLT antibody responses, as well as its capacity to adjuvant the immune responses for co-administered antigens. This Investigational Product requires special handling as a BSL-2 agent.

6.1.1 Acquisition

The dmLT vaccine will be provided by PATH Vaccine Solutions (PVS) and upon DMID authorization, will be transferred to the following address:

DMID Clinical Materials Services (CMS)
Fisher Bio Services
20439 Seneca Meadows Parkway
Germantown, MD 20876, U.S.A.
Tel: (240) 477-1350
Fax: (240) 477-1360
Email: DMID.CMS@thermofisher.com

Sodium bicarbonate, water for injection (WFI), sterile empty borosilicate glass vials for vaccine dilution and sterile normal saline for use in the study will be provided by the DMID CMS.

All study products will be shipped to the clinical research site upon request and approval from DMID.

6.1.2 Formulation, Packaging, and Labeling

6.1.2.1 dmLT

The bulk LT (R192G/L211A), or dmLT, was produced to current Good Manufacturing Practice (cGMP) specifications by IDT Biologika. The product, will be formulated as a freeze-dried (lyophilized), white to off-white cake, containing ~500 µg of dmLT in a sodium phosphate buffer supplemented with 5% lactose to stabilizer. The product will be provided in a 2 mL injection vial, 2 R, made of clear borosilicate glass, and sealed by a 13 mm dark grey rubber freeze-drying stopper and a crimped 13 mm aluminum cap, color: silver/red. Vial labels will include the cautionary statement: "Caution: New drug -Limited by Federal Law to investigational use".

6.1.2.2 Sodium Bicarbonate, USP

The sodium bicarbonate ($NaHCO_3$) is a white, crystalline powder that has a molecular weight of 84.01 gram/Mol and meets United States Pharmacopeia (USP) standards. This product comes in a 500 g plastic container and is manufactured under GMP guidelines. This product is used for the preparation and delivery of orally administered dmLT and for the placebo for the oral route of administration only. A label with the statement "Caution: New drug -Limited by Federal Law to investigational use" will be placed on the immediate package.

6.1.2.3 Water for Injection

The USP grade sterile water for injection (WFI) is nonpyrogenic and contains no bacteriostatic agent, or added buffer. It is supplied as single-dose container and should be used to **reconstitute** the lyophilized vaccine (dmLT) vial and to prepare the sodium bicarbonate buffer solution used for delivery of the orally administered dmLT. A label with the statement "Caution: New drug - Limited by Federal Law to investigational use" will be placed on the immediate package.

6.1.2.4 Sterile Normal Saline

The USP grade 0.9% Sodium Chloride or normal saline is a sterile, nonpyrogenic, isotonic solution; each mL contains sodium chloride 9 mg. It contains no bacteriostatic agent, antimicrobial agent, preservatives, or added buffer and is supplied only in single-dose containers. The solution may contain hydrochloric acid and/or sodium hydroxide for pH adjustment (pH 5.3, range 4.5-7.0). This product should be used to dilute the vaccine to the desired concentration and as the placebo for the sublingual and intradermal routes of administration only. A label with the statement "Caution: New drug -Limited by Federal Law to investigational use" will be placed on the immediate package.

6.1.3 Product Storage and Stability

The temperature of the storage unit must be manually recorded daily (excluding non-business days and holidays, as applicable) and continuously monitored and recorded during the course of this trial per the participating site standard operating procedures (SOPs), and documentation will be maintained. If the temperature fluctuates outside of the required range, the affected study product(s) must be quarantined at the correct storage temperature and labeled as 'Do Not Use' (until further notice). The participating site's research pharmacist must alert the site principal investigator and study coordinator, if the temperature fluctuates outside of the required range. In the event the temperature fluctuates outside of the required range, including accidental deep-freezing or disruption of the cold chain, the affected study product(s) must not be administered. The site principal investigator or responsible person should immediately contact the DMID Product Support Team at DMIDProductSupportTeam@niaid.nih.gov for further instructions before any additional study vaccinations are administered. Based on the information collected, DMID and/or the manufacturer will determine whether the affected study product(s) can be used. If it cannot be used, the site will receive specific instructions on how to return the affected study product(s) to the DMID CMS or destroy it on site. Additional instructions for quarantine and DMID contact information are provided in the protocol-specific MOP.

6.1.3.1 dmLT

The recommended storage temperature as marked on the label is ≤-10°C (-10°C or colder), which is also indicated in the Investigator's Brochure. Once rehydrated with sterile water for injection, as described in the pharmacy manual and preparation worksheets, dmLT should be held at 2-8˚C.

6.1.3.2 Sodium Bicarbonate, USP

The USP-grade sodium bicarbonate is preserved in well-closed containers. The Material Safety Data Sheet indicates that the product is stable under ordinary conditions of use and storage. The sodium bicarbonate is to be stored between 15°C and 30°C (59°F and 86°F).

6.1.3.3 Water for Injection (WFI)

The sterile water for injection must be stored at 20°C to 25°C (excursions between 15°C to 30°C are permitted according to USP, Controlled Room Temperature). Before use in dmLT reconstitution, WFI will be stored between 2°C and 8°C overnight (at least 18 hours) and alternatively may be stored at 2°C and 8°C for up to 18 months, not to exceed the product manufacturer expiry date.

6.1.3.4 Sterile Normal Saline

Sterile normal saline (0.9% Sodium Chloride, USP) must be stored at 20°C to 25°C (excursions between 15°C to 30°C are permitted according to USP, Controlled Room Temperature). Before use in dmLT dilutions, normal saline will be stored between 2°C and 8°C overnight (at least 18 hours) and alternatively may be stored at 2°C and 8°C for up to 18 months, not to exceed the product manufacturer expiry date.

6.2 Dosage, Preparation and Administration of Study Intervention/Investigational Product

Reconstitution of lyophilized dmLT. The vial containing the lyophilized dmLT product is to be reconstituted by the addition of 0.5 mL of cold WFIUSP Pharmaceutical grade, according to the directions in the vaccine preparation worksheet. The reconstituted vial, now containing dmLT at 1 mg/mL (or 1000 µg per mL), should be clear in appearance and the vial can be further diluted to specification. Once reconstituted (1 mg / mL), dmLT can be stored on wet ice or refrigerated for up to 6 hours. As per the DMID Guidelines for Clinical Study Product Management, written authorization from CPM/DMID for destruction of study products will be obtained before disposing reconstituted products (See MOP for further details).

Dilution of reconstituted dmLT. The desired dilutions of dmLT are then made, using cold sterile normal saline, according to the dosage to be administered and the route of administration. The dilution worksheets and details of storage, once dmLT vaccine has been diluted, are provided in the study-specific *Manual of Procedures*.

Preparation of Sodium Bicarbonate Buffer Solution (for Oral Route of Administration only). The sodium bicarbonate buffer solution should be prepared by the Investigational Pharmacist (or trained designee) and maintained at room temperature until used. Two grams (2 g) of sodium bicarbonate powder will be weighed and placed into an appropriate container (e.g., 150 mL bottle or larger). One hundred fifty mL (150 mL) of sterile WFI will then be added to the container and the solution mixed until crystals are completely dissolved.

Preparation of Sterile Normal Saline Placebo (for SL and ID Route of Administration only). The placebo will be prepared by withdrawing sufficient volume of sterile normal saline to administer a 0.1mL dose. Apply a new needle prior to administration for the ID route.

The vaccine or placebo by either route will be administered by a member of the research team who is licensed to administer medications or vaccines.

6.2.1 Oral Administration of dmLT

Participants will have nothing to eat or drink for 90 minutes before and after dosing. For oral routes of administration, the sodium bicarbonate buffer solution (2 g sodium bicarbonate in 150 mL of sterile WFI) is split into a 120 mL and 30 mL aliquot (e.g., into two disposable drinking cups). The designated amount of dmLT is transferred from the working dilution, as specified, into 30 mL of sodium bicarbonate buffer solution. Once the dmLT is added to the sodium bicarbonate buffer solution it must be administered within 15 minutes to the study participant. For oral vaccination, participants first ingest 120 mL of sodium bicarbonate buffer and then within 1-5 minutes ingest the designated dosage of dmLT, suspended in 30 mL sodium bicarbonate buffer solution. For the oral cohorts, participants that vomit or spit out study product within 90 minutes of taking the study product will be replaced.

6.2.2 Oral Administration of Placebo (Sodium Bicarbonate Buffer alone)

Participants will have nothing to eat or drink for 90 minutes before and after dosing. As with active vaccination, a sodium bicarbonate buffer solution will be prepared (2 g sodium bicarbonate in 150 mL of sterile WFI) and maintained at room temperature until use. To maintain the blind, participants first ingest 120 mL of the sodium bicarbonate buffer and then within 1-5 minutes ingest the remaining 30 mL of sodium bicarbonate buffer solution. For the oral cohorts, participants that vomit or spit out study product within 90 minutes of taking the study product will be replaced.

6.2.3 Sublingual Administration of dmLT

For SL routes of administration, participants will have nothing to eat or drink for 30 minutes before and after dosing. Participants will be asked to gargle and rinse their mouths for 10±5 minutes with bottled water or treated tap water that is supplied by study staff. After this is completed, a sterile gauze will be placed under the participant's tongue for ~1 minute ±15 seconds. Afterwards, the gauze will be removed, and the participant will receive the sublingual vaccine immediately. The designated amount of vaccine will be given in a 100 µL (0.1 mL) volume, diluted with normal saline, and be delivered underneath the tongue using a calibrated tuberculin or insulin syringe (or equivalent). After delivery of vaccine, participants will be instructed to tilt their head forward, chin to chest, for at least 1 minute. Afterwards, participants will be instructed to bring their head to normal position and swallow. Participants will be reminded not to eat, drink, or rinse the mouth for 30 minutes following dosing. For the SL cohorts, participants that vomit or spit out study product within 30 minutes of taking the study product will be replaced.

6.2.4 Sublingual Administration of Placebo (Normal Saline)

For SL routes of administration, participants will have nothing to eat or drink for 30 minutes before and after dosing. To maintain the blind, participants will undergo the same procedure as described above in Section 6.2.3 for the active SL vaccination, except 100 µL (0.1mL) of normal saline will be delivered under the tongue instead of active study product. For the SL cohorts, participants that vomit or spit out study product within 30 minutes of taking the study product will be replaced

6.2.5 Intradermal Administration of dmLT

For intradermal routes of administration, the arms of the participant will be examined for the most appropriate injection site, considering the avoidance of scars, tattoos, marks, etc. which may obscure or make the evaluation of local site reactogenicity difficult. Subsequent doses of vaccine, may be administered in the same arm or the arm may be switched as preferred by the participant. The designated amount of vaccine will be delivered in a single 100 µL (0.1 mL) volume by the ID route, using a tuberculin syringe (or equivalent) with a single-use sterile 25 gauge (or smaller) needle.

6.2.6 Intradermal Administration of Placebo (Normal Saline)

To maintain the blind, participants will have the same procedure as described above in Section 6.2.5 for the active ID vaccination, except 100 µL (0.1 mL) of normal sterile saline will be administered by the ID route instead of active study product.

6.3 Modification of Study Intervention/Investigational Product for a Participant

Not applicable

6.4 Accountability Procedures for the Study Intervention/Investigational Product(s)

The site principal investigator is responsible for ensuring study product distribution and disposition and has ultimate responsibility for study product accountability. The Investigator may delegate to the Site Research Pharmacist responsibility for study product accountability. Study product accountability records should include date received, date prepared, date administered, time of preparation, quantity administered, and the subject identification number to whom the study product was administered. The designated research pharmacist(s) will be responsible for maintaining accurate records of the shipments and dispensing the investigational products. The

pharmacy records must be available for inspection by the DMID monitor and is subject to inspection by a regulatory agency (e.g. FDA) at any time. Aliquots of dmLT dilutions in saline prepared for each cohort dosing level should be saved and stored at -80ºC±15°C for later shipment to PATH's analytical laboratory subcontractor for dmLT dose verification (see MOP for details on shipping instructions). Temporary storage on dry ice for up to 24 hours is allowed. The dose verification frozen aliquots may be stored as a reagent (i.e., does not need to be stored under the conditions of an investigational product). Used and unused study product (dmLT vials) will be retained and monitored until written authorization from the Study Sponsor (CPM/DMID) for destruction of study products is obtained. Final disposition of the unused study products will be determined by DMID and communicated to the participating sites by the DMID Clinical Project Manager.

6.5 Assessment of Subject Compliance with Study Intervention/Investigational Product

Participants will be vaccinated and observed for the respective time following dosing by a blinded member of the study team who is licensed to administer medications or vaccines. Observation times are approximately 90 minutes following oral dosing and 30 minutes following SL and ID dosing.

There will be no fasting requirement for the 30 minute observation period following ID dosing.

6.6 Concomitant Medications/Treatments

Participants are permitted to take birth control pills and vitamins throughout the course of the study; herbal supplements are not permitted. In addition, participants will be instructed that prescription or OTC anti-inflammatory medications will not be permitted in the 48 hours prior to receiving the investigational product. This includes medications that contain naproxen, aspirin, ibuprofen, and other non-steroidal anti-inflammatory drugs. All concomitant medications (e.g., vitamins, antacids, analgesics, prescription medications) taken 28 days prior to vaccination will be recorded in the concomitant medication data collection form. Any medications taken during the 7 days following a dose of vaccine are to be recorded on the participant's memory aid and/or reported to a research team member upon follow up clinic visit. All analgesics and prescription medications from 8 to 28 days post-vaccination will be recorded in the concomitant medication data collection form.

If the investigator learns that the participant has taken a prohibited medication (see inclusion/exclusion criteria) during the time immediately around the dosing of the investigational

product, the investigator will contact the DMID CPM and MO for instructions regarding the participant's continuation in the study.

7 STUDY SCHEDULE

7.1 Screening (Days -7 through -4)

Recruitment: Participants for this study will be recruited from the Mirpur field site. Study staff will visit nearby households of the Mirpur field clinic and have preliminary discussions with adults regarding the study, study procedures, and eligibility. During this process, study staff will identify potential participants for the study and invite them to come to the Mirpur field clinic at a scheduled time for a screening visit.

Research staff will obtain written consent per the standard informed consent process before conducting protocol-specific screening activities.

After signing the ICF, the following screening procedures will be performed:

- Confirm signed informed consent form.
- Collect demographic information, medical history, and concomitant medication history
- Record oral temperature, pulse, and blood pressure
- Perform an abbreviated physical examination, to include: head, eyes, ears, nose, throat (HEENT); lymph nodes; skin; pulmonary; cardiovascular; abdominal; neurological; and musculoskeletal systems
- Review eligibility criteria
- Collect approximately 2 mL of venous blood, for screening labs: complete blood counts (CBC) with differential for WBC, Hg, ANC, platelets count, creatinine, albumin, ALT, serum bilirubin, HBsAg, and HCV antibody
- Collect 13 mL of venous blood for cryopreservation of PBMC, for effector T cell assays
- Collect screening stool for culture and future use.
    - The study staff will provide a stool container to the potential participant and give instructions to collect stool. Participants will bring the stool sample back to the field clinic, within 8 hours of collection, for the completion of the screening stool for culture.
    - 1-2 gm of stool will be frozen at -80°C±15°C for future use microbiome studies

Prospective participants will be carefully screened to ensure that they are in excellent physical and mental health, and that they meet the eligibility criteria Screening tests will be conducted 4-7 days before Day 1 (the day of first vaccination with dmLT). All women will have a urine pregnancy test performed during screening and a urine pregnancy test on the vaccination days. To be eligible the result of the urine pregnancy test must be negative. During screening, participants will be informed of whether they will be in an oral, sublingual, or intradermal route

of administration cohort so that they are able to anticipate the required duration of fasting necessary prior to vaccination.

7.2 Enrollment/Baseline

7.2.1 VISIT 1- First Vaccination (Day 1)

- Confirm ongoing consent and review eligibility criteria prior to performing any study procedures, to include updating the medical history and concomitant medications
- Record oral temperature, pulse, and blood pressure
- Targeted physical examination, if indicated based on review of medical history
- Urine pregnancy test will be obtained from all women must be negative prior to dosing. Provide instructions to women participants to avoid pregnancy through the 28 days from receipt of the last dose of vaccine.
- Collect approximately 15 mL of venous blood:
  - 3 mL for serum ELISA and neutralization assays
  - 2 mL for ASC assay
  - 2 mL for ALS assay
  - 8 mL for cryopreservation of PBMC, for memory B cell assays
  - Collect plasma from PBMC tubes
- Collect stool (approximately 4 gm) and saliva (approximately 2 mL) for IgA assay.
- 1-2 gm of stool freeze at -80°C±15°C for future use microbiome studies
- Perform Pre-vaccination reactogenicity assessment. Note: the elements of the reactogenicity assessment will vary based on the route of administration of vaccine. The pre-vaccination assessment will serve as the baseline for the comparison of post-vaccination reactogenicity.
- Administer vaccine, per the randomization code and observing the required pre-vaccination fasting for oral and rinsing for SL dosing
- Provide memory aid and other 7-day solicited AE and reactogenicity instructions
- Provide digital thermometer for recording of daily oral temperature for seven days following administration of study product. Participants will be instructed on the use of the digital thermometer.
- Perform post-vaccination reactogenicity assessment. Participants will be examined at the end of a 30 minute observation period following each vaccination for the SL and ID cohorts, and at the end of a 90 minute observation period for oral cohorts.

- Reactogenicity Assessments: Will include brief history for assessment of AE/SAEs just prior to and following vaccination (Days 1, 15, and 29 for oral/SL; Days 1, 22, and 43 for ID), which includes an assessment of systemic and local reactions according to section 9.1.

- Provide stool sample collection container and instructions on how to collect stool sample
- Confirm date and time for Visit 2

7.3 Follow-up

7.3.1 VISIT 2 (~1 week post-dose 1± 1 day)

- Confirm ongoing consent prior to performing any study procedures. Conduct eligibility review. Review any AEs or SAEs that have occurred since the last visit as well as update the medical history and concomitant medications as necessary. Assess for injection site necrosis within 7 days of vaccination.
  - Assess for urticaria, laryngospasm, bronchospasm, or anaphylaxis determined to be related to vaccine and within 7 days of vaccination.
  - Assess for participants experience facial nerve neuropathy, confirmed by an investigator and determined to be related to vaccine.
    - Record oral temperature, pulse, and blood pressure
    - Targeted physical examination, if indicated based on review of medical history
    - Collect approximately 8 mL of venous blood:
      - 2 mL for serum ELISA and neutralization assays
      - 2 mL for ASC assay
      - 2 mL for ALS assay
      - 2 mL for ASC with homing
      - Collect plasma from PBMC tubes
- Collect stool (approximately 4 gm) and saliva (approximately 2 mL) for IgA assay.
- 1-2 gm of stool frozen at -80°C±15°C for future use microbiome studies
- Collect memory aid/reactogenicity information
- Provide stool sample collection container and instructions on how to collect stool sample
- Provide instructions to women participants to avoid pregnancy through the 28 days from receipt of the last dose of vaccine.
- Confirm date and time for Visit 3

7.3.2 VISIT 3 - Second Vaccination (~14 days post dose 1 ±2 days)

- This visit is ~14 days after the first dose for oral and SL cohorts and ~21 days after the first dose for the ID cohorts (window ±2 days)
- Confirm ongoing consent and review eligibility criteria with the participant prior to performing any study procedures, to include updating the medical history and concomitant medications
- Record oral temperature, pulse, and blood pressure
- Targeted physical examination, if indicated based on review of medical history
- Collect approximately 6 mL of venous blood:
  - 2 mL for serum ELISA and neutralization assays
  - 2 mL for ASC assay
  - 2 mL for ALS assay
- Collect stool (approximately 4 gm) and saliva (approximately 2 mL)
- 1-2 gm of stool frozen at -80°C±15°C for future use microbiome studies
- Urine pregnancy test for all women will be performed and must be negative prior to dosing. Provide instructions to women participants to avoid pregnancy through the 28 days from receipt of the last dose of vaccine.
- Perform pre-vaccination reactogenicity assessment
- Administer vaccine dose 2, per the randomization code and observing the required pre-vaccination fasting for oral and rinsing for SL dosing
- Provide memory aid and other 7-day solicited AE and reactogenicity instructions
- Ask participant if they still have the digital thermometer for recording daily oral temperature for seven days following administration of the study product and offer re-instruction on the use of the thermometer as needed.
- Perform post-vaccination reactogenicity assessment after 90 minutes for oral cohorts and after 30 minutes for SL and ID cohorts. This assessment is to include unsolicited AEs and SAEs.
- Provide stool sample collection container and instructions on how to collect stool sample
- Confirm date and time for Visit 4

7.3.3 VISIT 4 (~1 week post-dose 2±1day)

- Confirm ongoing consent prior to performing any study procedures. Conduct eligibility review. Review any AEs or SAEs that have occurred since the last visit as well as update the medical history and concomitant medications as necessary
  - Assess for injection site necrosis within 7 days of vaccination.

- Assess for urticaria, laryngospasm, bronchospasm, or anaphylaxis determined to be related to vaccine and within 7 days of vaccination.
- Assess for participants experience facial nerve neuropathy, confirmed by an investigator and determined to be related to vaccine.

- Record oral temperature, pulse, and blood pressure
- Targeted physical examination, if indicated based on review of medical history
- Collect approximately 21mL of venous blood:
  - 2 mL for serum ELISA and neutralization assays
  - 2 mL for ASC assay
  - 2 mL for ALS assay
  - 2 mL for ASC with homing
  - 13mL for cryopreservation of PBMC, for memory B cell (5 mL) and effector T cell assays (8 mL)
  - Collect plasma from PBMC tubes
- Collect stool (approximately 4 gm) and saliva (approximately 2 mL) for IgA assay.
- 1-2 gm of stool frozen at -80°C±15°C for future use microbiome studies
- Collect memory aid/reactogenicity information
- Provide stool sample collection container and instructions on how to collect stool sample
- Provide instructions to women participants to avoid pregnancy through the 28 days from receipt of the last dose of vaccine.
- Confirm date and time for Visit 5

7.3.4 VISIT 5 - Third Vaccination (~14 days post dose-2 ±2 days)

- This visit is ~14 days after the second dose for oral and SL cohorts and ~21 days after the second dose for the ID cohorts (window ±2 days)
- Confirm ongoing consent and review eligibility criteria with the participant prior to performing any study procedures, to include updating the medical history and concomitant medications
- Record oral temperature, pulse, and blood pressure
- Targeted physical examination, if indicated based on review of medical history
- Collect approximately 6 mL of venous blood:
  - 2 mL for serum ELISA and neutralization assays
  - 2 mL for ASC assay
  - 2 mL for ALS assay
- Collect stool (approximately 4 gm) and saliva (approximately 2 mL) for IgA assay.

- 1-2 gm of stool frozen at -80°C±15°C for future use microbiome studies
- Urine pregnancy test for women will be performed and must be negative prior to dosing. Provide instructions to women participants to avoid pregnancy through the 28 days from receipt of the last dose of vaccine.
- Perform Pre-vaccination reactogenicity assessment
- Administer vaccine dose 3, per the randomization code and observing the required pre-vaccination fasting for oral and rinsing for SL dosing
- Provide memory aid and other 7-day solicited AE and reactogenicity instructions
- Ask participant if they still have the digital thermometer for recording daily oral temperature for seven days following administration of the study product and offer re-instruction on the use of the thermometer as needed.
- Perform post-vaccination reactogenicity assessment after 90 minutes for oral cohorts and after 30 minutes for SL and ID cohorts. This assessment is to include unsolicited AEs and SAEs.
- Provide stool sample collection container and instructions on how to collect stool sample
- Confirm date and time for Visit 6

7.3.5 VISIT 6 (~1 week post-dose 3±1 day)

- Confirm ongoing consent prior to performing any study procedures. Conduct eligibility review. Review any AEs or SAEs that have occurred since the last visit as well as update the medical history and concomitant medications as necessary
  - Assess for injection site necrosis within 7 days of vaccination.
  - Assess for urticaria, laryngospasm, bronchospasm, or anaphylaxis determined to be related to vaccine and within 7 days of vaccination.
  - Assess for participants experience facial nerve neuropathy, confirmed by an investigator and determined to be related to vaccine.
- Record oral temperature, pulse, and blood pressure
- Targeted physical examination, if indicated based on review of medical history
- Collect approximately 22 mL of venous blood:
  - 2 mL for clinical safety labs: ALT (also known as SGPT), total bilirubin, and creatinine
  - 2 mL for serum ELISA and neutralization assays
  - 2 mL for ASC assay
  - 2 mL for ALS assay
  - 2 mL for ASC with homing

- 12 mL for cryopreservation of PBMC, for memory B cell (5 mL) and effector T cell assays (7 mL)
- Collect plasma from PBMC tubes

- Collect stool (approximately 4 gm) and saliva (approximately 2 mL) for IgA assay.
- 1-2 gm of stool frozen at -80°C±15°C for future use microbiome studies
- Collect memory aid/reactogenicity information
- Provide stool sample collection container and instructions on how to collect stool sample
- Provide instructions to women participants to avoid pregnancy through the 28 days from receipt of the last dose of vaccine.
- Confirm date and time for Visit 7

7.3.6 VISIT 7 (~4 weeks post-dose 3 (window ±3 days)

- Confirm ongoing consent prior to performing any study procedures. Conduct eligibility review. Review any AEs or SAEs that have occurred since the last visit as well as update the medical history and concomitant medications as necessary
  - Assess for injection site necrosis within 7 days of vaccination.
  - Assess for urticaria, laryngospasm, bronchospasm, or anaphylaxis determined to be related to vaccine and within 7 days of vaccination.
  - Assess for participants experience facial nerve neuropathy, confirmed by an investigator and determined to be related to vaccine.
- Record oral temperature, pulse, and blood pressure
- Targeted physical examination, if indicated based on review of medical history
- Collect approximately 17mL of venous blood:
  - 2 mL for clinical safety labs: CBC with differential for WBC, Hg, ANC, platelets; creatinine, albumin, and ALT
  - 3 mL for serum ELISA and neutralization assays
  - 12 mL for cryopreservation of PBMC, for memory B cell (5 mL) and effector T cell assays (7 mL)
  - Collect plasma from PBMC tubes
- Collect stool (approximately 4 gm) and saliva (approximately 2 mL) for IgA assay.
- 1-2 gm of stool frozen at -80°C±15°C for future use microbiome studies
- Provide stool sample collection container and instructions on how to collect stool sample
- Provide instructions to women participants to avoid pregnancy through the 28 days from receipt of the last dose of vaccine.
- Confirm date and time for Visit 8

7.3.7 VISIT 8 (~12weeks post-dose 3 (window ±1week)

- Confirm ongoing consent prior to performing any study procedures. Conduct eligibility review. Review any SAEs that have occurred since the last visit as well as update the medical history and concomitant medications as necessary, to support an SAE.
- Record oral temperature, pulse, and blood pressure
- Targeted physical examination, if indicated based on review of medical history
- Collect approximately 15 mL of venous blood:
  - 2 mL for serum ELISA and neutralization assays
  - 13 mL for cryopreservation of PBMC, for memory B cell (5 mL) and effector T cell assays (8 mL)
  - Collect plasma from PBMC tubes
- Confirm date and time for Visit 9

7.4 Final Study Visit

7.4.1 VISIT 9 (~6 months post-dose 3, window ±2 weeks)

- Confirm ongoing consent prior to performing any study procedures. Review any SAEs that have occurred since the last visit as well as update the medical history and concomitant medications as necessary, to support an SAE.
- Record oral temperature, pulse, and blood pressure
- Collect approximately 15 mL of venous blood:
  - 15 mL for cryopreservation of PBMC, for memory B cell (5 mL) and effector T cell assays (10mL)
  - Collect plasma from PBMC tubes

7.5 Early Termination Visit

Upon voluntary withdrawal of participation or any other reason for early termination from the study attempt to complete a clinic visit to include:

- Obtain an interim medical history and concomitant medication information and record on the appropriate case report form.
- Assess reactogenicity and review memory aid if early termination visit occurs within 7 days of any vaccination.
- Assess for unsolicited AEs if visit occurs within 28 days of third vaccination

- Assess for SAE during all early termination visits. Participants will be asked to be followed until resolution of any AEs.
- Record oral temperature, pulse, and blood pressure
- Perform a targeted physical examination, if indicated based on review of medical history
- Obtain serum samples for laboratory safety assays if visit occurs ≤28 days after third vaccination

7.6 Unscheduled Visit

If an unscheduled visit occurs, a member of the clinical study team (PI, Co-Investigator, Study Coordinator, or Clinical Nurse) will interview and evaluate the participant to determine the cause of the visit and provide care as needed, and information will be documented on a supplemental visit eCRF.

Any of the following activities may be performed during this visit:

- Review of reactogenicity (if within 7 days of last vaccination), unsolicited non-serious AE information (if within 28 days of last vaccination), or any SAE information (anytime within study)
- Review of concomitant medications (if within 28 days of last vaccination)
- Obtain interim medical history
- Targeted physical examination, if indicated
- If the unscheduled visit is for eligible diarrhea (section 8.2.2), then a stool specimen will be collected for culture.
- If the unscheduled visit is grade 3 local injection site reactogenicity event in a participant in the ID cohort and the event is within 7 days of a dose administration (as described at the end of section 8.1), then measurements of the reaction and a digital photo will be performed. Unscheduled daily visits by the study team will continue until the injection site reaction is deemed stable or returns to a grade 2 or lower severity.
- Upon the judgement of the clinical investigator, clinical laboratory assessments may be performed to evaluate an AE and medications (e.g., analgesics) may be recommended to treat an AE

8 STUDY PROCEDURES/EVALUATIONS

8.1 Clinical Evaluations

Medical History: Will be obtained by interview of the participants. Participants will be queried regarding a history of significant medical disorders of the head, eyes, ears, nose, throat (HEENT), mouth, cardiovascular system, lungs, gastrointestinal tract, liver, pancreas, kidney, urologic, nervous system, blood, lymph glands, endocrine system, musculoskeletal system, skin, and genital/reproductive tract. A history of any allergies, cancer, immunodeficiency, psychiatric illness, substance abuse, and autoimmune disease will be solicited.

Medication History: All concomitant medications (e.g., vitamins, antacids, analgesics, prescription medications) taken 28 days prior to vaccination will be recorded in the concomitant medication data collection form. Any medications taken during the 7 days following a dose of vaccine are to be recorded on the participant's memory aid and/or reported to a research team member upon follow up clinic visit. All analgesics and prescription medications from 8 to 28 days post-vaccination will be recorded in the concomitant medication data collection form. Assessment of eligibility also will include a review of permitted and prohibited medications (per the exclusion criteria).

Physical Examination: At screening (Days -7 through -4) appropriate study personnel will record oral temperature, pulse and blood pressure and perform an abbreviated physical examination to assess general wellness which will include the following areas/systems: HEENT; lymph nodes; skin; pulmonary; cardiovascular; abdominal; neurological; and musculoskeletal systems. The physical examination should specifically address issues identified by the medical history of the participant. At following visits including enrollment, oral temperature, pulse, and blood pressure will be recorded and a targeted physical examination may be conducted if indicated based on review of medical history.

Reactogenicity Assessments: Will include brief history for assessment of AE/SAEs just prior to and following vaccination (Days 1, 15, and 29 for oral/SL; Days 1, 22, and 43 for ID), which includes an assessment of systemic and local reactions according to section 9.1. Participants will be examined at the end of a 30 minute observation period following each vaccination for the SL and ID cohorts, and at the end of a 90 minute observation period for oral cohorts.

Memory Aids: All participants will complete a subject memory aid distributed after each vaccination. Subject memory aids will be reviewed with the participant for solicited AEs. Participants will be instructed to record daily oral temperature on the memory aid. Participants will be asked about the occurrence of unsolicited AE/SAEs during visits 2 (Day 8, oral/SL/ID), 4

(Day 22, oral/SL; Day 29, ID), and 6 (Day 36, oral/SL; Day 50, ID). Following review, the appropriate source document and electronic case report form (eCRF) will be completed by the study staff. For illiterate participants, either a literate member of the household will be trained to help to fill out the memory aid or a field staff member will be identified to visit the household to help with the completion of the memory aid. Memory aids will not be retained as a source document.

Adverse Event Review: It is not anticipated that AEs of diarrhea and/or vomiting (or other signs of enterotoxicity) will be common or severe in this study, based on the previous experience with oral and SL dmLT or ID dmLT clinical trials. However, an evaluation of any participant who experiences diarrhea will be performed by the study staff, if it occurs within 7 days of dosing. The determination of severity diarrhea is based as follows:

Clinical Sign	Normal	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)
Diarrhea	Normal or 1-2 loose stool per 24 hours	3 loose stools per 24 hours	4-7 loose stools per 24 hours	8 or more loose/watery stools per 24 hours or require outpatient IV hydration, ED visit or hospitalization for diarrhea

The assessment of dehydration will be performed in clinic by a study investigator, according to Table 2 below. The management of dehydration will be guided according to Table 2 below, but is ultimately to be managed at the discretion of the investigator. The assessment of severity of diarrhea and level of dehydration and treatment will be documented in the appropriate source document. A description for the conditions leading to the performance of stool cultures is described in section 8.2.

Table 2: Assessment of Dehydration and Treatment Guidance

Clinical Assessment	General Appearance	Well, Alert	Restless, Irritable	Lethargic or Unconscious
	Eyes	Normal	Sunken	Sunken
	Thirst	Normal	Thirsty, drinks eagerly	Unable to Drink
	Skin Turgor	Normal	Slow Return	Very Slow Return
Degree of Dehydration		No signs of dehydration	Some Dehydration	Severe Dehydration
Treatment Guidance		Reassure and educate on signs of dehydration	Provide ORS and reassess after ORS completed, until no dehydration	Provide ORS and consider IVF, reassess and repeat until no dehydration

ORS = oral rehydration solution
IVF = intravenous fluid

For participants in the ID cohorts, should there be any grade 3 local injection site reactogenicity (i.e., within 7 days of dose administration); the research staff will request the participant be evaluated by the study team to mark the widest edges of the respective reaction (e.g., redness, induration, or bruising), measure, and record on the CRF. Furthermore, a digital photo of the site will be taken by the study team. Additional measurements and a photo should be taken on a daily basis by the study team until the injection site reaction is deemed stable or returns to a grade 2 or lower severity.

8.2 Laboratory Evaluations

8.2.1 Clinical Laboratory Evaluations

A total of 2 mL blood will be collected for screening clinical labs for the evaluation of eligibility. If screening laboratory results are out of range value, they may be repeated once provided there is an alternative explanation for the out of range value. Participants must have acceptable screening laboratory findings within 7 days prior to enrollment. The acceptable ranges for study eligibility are listed in *Appendix B*.

For the assessment of safety, a total of 2 mL of blood will be collected for clinical safety labs on visit 7, about 4 weeks after the third dose of vaccine. These include a CBC with differential for WBC, Hg, ANC, Platelet count, creatinine, albumin, and ALT. In addition, 2 mL of blood will be collected at visit 6 (7-days post-third dose of vaccine) for ALT, serum bilirubin, and creatinine. The grading of toxicity for clinical safety lab values that are not in the normal range is

provided in *Appendix C*.

All women will have a urine pregnancy test performed during screening and a urine pregnancy test on the vaccination days. The results of the urine pregnancy test must be negative prior to enrollment in the study, and prior to each vaccination.

For those laboratory tests run as part of a panel of tests, but not required by the protocol, the site is required to do the following:

- Enter all non-required lab results into the database in the comments section of the eCRF.
- When a result is abnormal per the site normal laboratory reference range, the result must be:
    - Entered in the comments section of the database including assessment for clinical significance by site PI,
    - An AE generated and followed until resolution if clinically significant based on assessment by the site PI,
    - If clinically significant, graded according to either the toxicity tables in Appendix C or if a grading scale is not available, the PI should use the guidance for AE grading.

8.2.2 Clinical Microbiology Evaluation

For any participant who experiences any diarrhea within 3 days (72 hours) of receipt of any dose of study product will be requested to provide a stool specimen for stool culture.

For any participant (irrespective of the route of administration) that experiences moderate or severe diarrhea within 28 days of receipt of any dose of study product will be requested to provide a stool specimen for stool culture. If the participant experiences further episodes of moderate or severe diarrhea, there will be a maximum of one stool culture to be performed per 7 day period.

Upon notification of eligible diarrhea, study staff will instruct participants to come to the research clinic to collect a stool specimen or a study staff member will go to the household of the participant for the collection of a stool specimen. The stool will be cultured, using standard bacteriological methods, for the presence of: ETEC, *Vibrio cholerae*, *Shigella* sp., *Salmonella* sp., and *Campylobacter* sp.

8.2.3 Special Assays or Procedures

Detailed procedures for the conduct of each of the immunology assays are described in the *Manual of Procedures*.

The following immunology assays will be performed at icddr,b (Dhaka, Bangladesh):

Table 3: Assays to be performed at icddr,b

Immunology Assay:	Visit(s) collected at:
Serum anti-dmLT IgG and IgA by ELISA	Visits 1, 2, 3, 4, 5, 6, 7, and 8
dmLT-specific IgG and IgA ASC assays	Visits 1, 2, 3, 4, 5, and 6
dmLT-specific IgG and IgA ALS assays	Visits 1, 2, 3, 4, 5, and 6
ASC homing studies	Visits 2, 4, and 6
Fecal total IgA and anti-dmLT IgA by ELISA	Visits 1, 2, 3, 4, 5, 6 and 7
Saliva total IgA and anti-dmLT IgA by ELISA	Visits 1, 2, 3, 4, 5, 6 and 7
dmLT-specific memory B cell responses	Visits 1, 4, 6, 7, 8, and 9

The following immunology assay will be performed at CVD (Baltimore, MD, U.S.):

- Serum toxin (LT) neutralization assay
- dmLT-specific effector and memory T cell responses, by CyTOF--including the characterization of: homing potential, cytokine/chemokine production profile, degranulation capacity, and activation potential
    - Collected at visits screening, 4, 6, 7, 8, and 9

8.2.4 Specimen Preparation, Handling, and Shipping

All sample collection time are described in the Table 3 and Appendix A.

8.2.4.1 Instructions for Specimen Preparation, Handling, and Storage

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

8.2.4.2 Specimen Shipment

Instructions for specimen shipping are described in the *Manual of Procedures*.

9 ASSESSMENT OF SAFETY

9.1 Specification of Safety Parameters

The Investigator is responsible for reporting all AEs that are observed or reported during the study, regardless of their relationship to investigational product or their clinical significance.

All events will be recorded on appropriate clinical report forms with notation of duration, severity, and outcome.

Safety and tolerability will be assessed by the frequency and severity of:

1. Study vaccine-related SAEs occurring from first vaccination through 6 months after the last study vaccination.

2. Solicited AEs – reactogenicity events occurring within 7 days of each dose of vaccine:

    a. Systemic Reactions to include: feverishness (chills/shivering/sweating), fatigue (tiredness), malaise (general unwell feeling), myalgia (body aches, muscular pain), or headache, diarrhea, nausea, vomiting, or abdominal discomfort. Note: for the oral and SL routes of administration, diarrhea, nausea, vomiting, and abdominal discomfort will be evaluated as local reactions as described below.

    b. Systemic Reactions to also include the measurement of daily oral temperature

    c. Local Reactions

        i. For oral route of administration: irritation of the oral cavity or tongue, plus diarrhea, nausea, vomiting, and abdominal discomfort

        ii. For SL route of administration: irritation of the oral cavity or tongue, or facial nerve disturbance, plus diarrhea, nausea, vomiting, and abdominal discomfort

        iii. For ID route of administration: injection site pain, redness, swelling, bruising, itching, hypo/hyper pigmentation and induration, vesicles or hardened mass.

3. Unsolicited AEs – study vaccine-related non-serious AEs from first vaccination through 28 days after the last (third) dose of vaccine

4. Withdrawals and discontinuation of study vaccinations will be assessed as a part of determining tolerability

9.2 Methods and Timing for Assessing, Recording, and Analyzing Safety Parameters

9.2.1 Adverse Events

Adverse Event (AE): International Conference on Harmonization (ICH) E6 defines an AE as any untoward medical occurrence in a patient or clinical investigation participants administered a pharmaceutical product regardless of its causal relationship to the study treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product. The occurrence of an AE may come to the attention of study personnel during study visits and interviews of a study recipient presenting for medical care, or upon review by a study monitor.

AEs, including local and systemic (subjective and quantitative) reactions, not meeting the protocol-defined criteria for SAEs will be captured on the appropriate data collection form and eCRF. Information to be collected for unsolicited non-serious AEs includes event description, date of onset, licensed study physician's assessment of severity and relationship to study product and alternate etiology (if not related to study product) (assessed only by those with the training and authority to make a diagnosis and listed on the Form FDA 1572 as the site principal investigator or sub-investigator), date of resolution of the event, seriousness and outcome. AEs occurring during the collection and reporting period will be documented appropriately regardless of relationship. AEs will be followed through resolution.

Any medical condition that is present at the time that the participant is screened will be considered as baseline and not reported as an AE. However, if the severity of any pre-existing medical condition increases, it will be recorded as an AE.

AEs must be graded for severity and assessed for relationship to study product (see definitions below). Adverse events characterized as intermittent require documentation of onset and duration of each episode. The start and stop date of each reported AE will be recorded on the appropriate data collection form and eCRF.

FDA defines an AE as any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related.

Severity of Event: AEs will be assessed by a licensed study physician listed on the Form FDA 1572 as the site principal investigator or appropriate sub-investigator the "Laboratory and

Clinical Toxicity Grading Scales" in *Appendix C*. For events not included in the protocol-defined grading system, the following guidelines will be used to quantify severity:

- Mild (Grade 1): Events require minimal or no treatment and do not interfere with the participant's daily activities.
- Moderate (Grade 2): Events result in a low level of inconvenience or concern with therapeutic measures. Moderate events may cause some interference with functioning and daily activities.
- Severe (Grade 3): Events interrupt the participant's usual daily activities and may require systemic drug therapy or other treatment. Severe events are usually incapacitating.

Relationship to Study Product: The study physician's assessment of an AE's relationship to study product is part of the documentation process, but it is not a factor in determining what is or is not reported in this study. If there is any doubt as to whether a clinical observation is an AE, the event should be reported. The relationship to study product must be assessed for AEs using the terms: related or not related. In a clinical trial, the study product must always be suspect. To help assess, the following guidelines are used:

- Related – There is a reasonable possibility that the study product caused the adverse event. Reasonable possibility means that there is evidence to suggest a causal relationship between the study product and the adverse event.
- Not Related – There is not a reasonable possibility that the administration of the study product caused the event.

9.2.2 Solicited Events

The Toxicity Grading Scales to be used to guide the grading of solicited AEs is in *Appendix C*.

9.2.3 Serious Adverse Events

Serious Adverse Event (SAE): An adverse event or suspected adverse reaction is considered "serious" if, in the view of either the site principal investigator or sponsor, it results in any of the following outcomes:

- Death,
- a life-threatening adverse event*,
- inpatient hospitalization (defined by local practice as the study participant being present in the hospital greater than 24 hours) or prolongation of existing hospitalization,

- a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions, or
- a congenital anomaly/birth defect.
- Important medical events that may not result in death, be life-threatening, or require hospitalizations may be considered serious when, based upon appropriate medical judgment they may jeopardize the patient or participant 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.

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

SAEs will be:

- Assessed for severity and relationship to study product and alternate etiology (if not related to study product) by a licensed study physician listed on the Form FDA 1572 as the site principal investigator or sub-investigator.
- Recorded on the appropriate SAE forms and the eCRF.
- Followed through resolution by a licensed study physician listed on the Form FDA 1572 as the site principal investigator or sub-investigator.
- Reviewed and evaluated by an Independent Safety Monitor (ISM), Independent Protocol Safety Team (IPST), both the DMID DSMB and local DSMB (periodic review unless related), DMID, and the IRB.

9.2.4 Procedures to be followed in the Event of Abnormal Laboratory Test Values or Abnormal Clinical Findings

The site principal investigator or appropriate sub-investigator is responsible for recording all AE/SAEs that are observed or reported during this study, regardless of the relationship to study product. AE/SAEs or abnormal clinical findings will be collected, assessed, documented, reported, and followed until resolved or considered stable.

9.3 Reporting Procedures

9.3.1 Serious Adverse Events

If in the opinion of a study physician Investigator the event meets the criteria of a SAE, the following procedures will occur:

All SAEs will be:

- recorded on the appropriate AE eCRF, DMID SAE form, and icddr,b SAE form
- followed by a study physician until satisfactory resolution or until the PI or co-Investigator deems the event to be chronic or the participant to be stable as reviewed by a study physician

Any AE that meets a protocol-defined serious criterion must be submitted immediately (within 24 hours of site awareness) on the DMID SAE form to the DMID Pharmacovigilance Group at the following address:

DMID Pharmacovigilance Group
Clinical Research Operations and Management Support (CROMS)
6500 Rock Spring Dr. Suite 650
Bethesda, MD 20817, USA
SAE Hot Line: 1-800-537-9979 (US) or 1-301-897-1709 (outside US)
SAE FAX: 1-800-275-7619 (US) or 1-301-897-1710 (outside US)
SAE Email Address: PVG@dmidcroms.com

In addition to the SAE form, selected SAE data fields must also be entered into Advantage eClinical[SM]. Please see the protocol-specific MOP for details regarding this procedure.

Other supporting documentation of the event may be requested by the DMID Pharmacovigilance Group and should be provided as soon as possible.

The site will send a copy of the SAE report(s) to the ISM when they are provided to the DMID Pharmacovigilance Group. The DMID Medical Monitor and DMID Clinical Project Manager will be notified of the SAE by the DMID Pharmacovigilance Group. The DMID Medical Monitor will review and assess the SAE for regulatory reporting and potential impact on study participant safety and protocol conduct.

At any time after completion of this study, if the site principal investigator or appropriate sub-investigator becomes aware of an SAE that is suspected to be related to study product, the site principal investigator or appropriate sub-investigator will report the event to the DMID Pharmacovigilance Group.

In addition, such events will be reported to the local IRB/ERC in accordance with IRB/ERC policy.

All SAEs will be followed until satisfactory resolution or until the PI or Co-Investigator deems the event to be chronic or the participant to be stable.

9.3.2 Regulatory Reporting for Studies Conducted Under DMID-Sponsored IND

Following notification from the investigator, DMID, the IND sponsor, will report any suspected adverse reaction that is both serious and unexpected. DMID will report an AE as a suspected adverse reaction only if there is evidence to suggest a causal relationship between the study product and the adverse event. DMID will notify FDA and all participating investigators (i.e., all investigators to whom the sponsor is providing drug under its INDs or under any investigator's IND) in an IND safety report of potential serious risks from clinical trials or any other source, as soon as possible, but in no case later than 15 calendar days after the sponsor determines that the information qualifies for reporting as specified in 21 CFR Part 312.32. DMID will also notify FDA of any unexpected fatal or life-threatening suspected adverse reaction as soon as possible but in no case later than 7 calendar days after the sponsor's initial receipt of the information. Relevant follow-up information to an IND safety report will be submitted as soon as the information is available. Upon request from FDA, DMID will submit to FDA any additional data or information that the agency deems necessary, as soon as possible, but in no case later than 15 calendar days after receiving the request. All serious events designated as "not related" to study product(s), will be reported to the FDA at least annually in a summary format.

9.3.3 Reporting of Pregnancy

Reproductive animal toxicity studies have not been conducted with dmLT vaccine. Therefore, the risk to the unborn fetus or pregnant mother is unknown. Consequently, all women will be instructed to avoid pregnancy through the 28 days from receipt of the last dose of vaccine. Female participants must be non-pregnant at enrollment and prior to the receipt of each dose of vaccine. *All women presenting for screening will have urine pregnancy testing. "Females of childbearing potential must agree to use an efficacious hormonal or barrier method of birth control from screening and through 28 days post last dose of vaccine. Abstinence is also acceptable."*

If an eligible female participant has a positive urine pregnancy test at any time, she will not receive any further study treatment. Should a female participant become pregnant subsequent to Dose 1, the Investigator will obtain permission from the participant to follow the participant's pregnancy. If permission is obtained, the Investigator should report to DMID the course of pregnancy including perinatal and neonatal outcome on the appropriate eCRF.

Although pregnancy is not a SAE, all pregnancies occurring during this study will be reported in the same time frame as SAEs. The Investigator will immediately notify the DMID MM and CPM about the pregnancy and complete a Pregnancy Report eCRF. If an SAE occurs during the pregnancy, the SAE will be reported on the appropriate SAE form and faxed to DMID-CROMS if appropriate.

9.4 Type and Duration of Follow-up of Subjects after Adverse Events

All AEs and SAEs will be followed until resolved or considered stable.

9.5 Halting Rules

Further dosing of any participant only for the route (oral, SL, ID) will be halted if any of the following criteria are met. The study will be halted, and all further vaccine administration will be halted for DSMB review/recommendation if any of the following criteria are met for each route of administration:

- Any participant experiences vaccine-related SAE (e.g., excluding trauma or accident) from the time of the first study vaccination through the participant's last study visit.
- Any participant experiences an injection site necrosis within 7 days of vaccination.
- Any participant experiences an anaphylactic reaction related to vaccine occurring within 24 hours after vaccination.
- Any participant experiences urticaria, laryngospasm, bronchospasm, or anaphylaxis determined to be related to vaccine and within 7 days of vaccination.
- Any participant experiences facial nerve neuropathy, within 28 days of vaccination, confirmed by an investigator and determined to be related to vaccine.
- Any participant experiences a death for any reason excluding trauma or accident.

In addition, the study will be halted if two or more participants in the same cohort or three participants across all cohorts within a route of administration (who received at least one dose of study product) experience:

- The same severe (Grade 3) study vaccine-related local solicited event,
- The same severe (Grade 3) study vaccine-related systemic solicited event,
- The same severe (Grade 3) study vaccine-related clinical laboratory test,
- The same severe (Grade 3) study vaccine-related vital sign, or
- Any other severe (Grade 3) AE of the same system organ class

Exceptions to this would be:

- If there are obvious and acceptable physiological explanation for a Grade 3 abnormality (example, grade 3 hematuria in menstruating females)

Once a halting rule is met, further dosing of the respective route will cease, but follow-up visits may continue. An *ad hoc* DMID DSMB meeting and local DSMB meeting will be convened to discuss the halting event. If, following review by both the DMID DSMB and local DSMB, this is deemed acceptable to restart the study, the study will resume, upon DMID's approval and authorization.

9.6 Safety Oversight

There will be two independently functioning Data and Safety Monitoring Boards (DSMB) which will provide safety oversight for this study.

9.6.1 DMID DSMB

The DMID DSMB is convened by authority of DMID and is advisory to DMID and the study team. The DMID DSMB must consist of at least three voting members including a biostatistician experienced in statistical methods for clinical trials and a clinician with relevant expertise. Selection of DMID DSMB members should include consideration of clinical trials experience, relevant expertise, prior DSMB service and absence of significant conflict of interest. DMID is responsible for deciding whether consultancies or the financial interests of the members materially affect their objectivity. Members will notify DMID promptly if a change occurs that may create a potential conflict of interest.

The DMID DSMB will operate under the rules of a DMID-approved charter that will be reviewed and finalized following the organizational meeting of the DSMB. The DMID DSMB Charter serves as the standard operating procedure and defines the primary responsibilities of the DSMB, its membership, the purpose and timing of its meetings, data to be reviewed, and procedures for ensuring confidentiality and proper communication. The following times are the proposed scheduled meetings of the DMID DSMB:

- First/Organizational DMID DSMB meeting –The first meeting of the DMID DSMB will be primarily organizational and to review the study protocol, safety data shells, and frequency of scheduled meetings. This meeting will occur prior to the initiation of study enrollment.

  Second Scheduled DMID DSMB meeting – to review the available safety data through 7 days post-third dose of vaccine for cohorts A-F prior to progressing to cohorts G-I, and make a recommendation whether to proceed.

- Third Scheduled DMID DSMB meeting – to review to aggregate safety data through 28 days post-third dose of vaccine for all nine cohorts (can be before database lock).
- Final DMID DSMB meeting - 6 to 8 months after clinical database lock to review the cumulative unblinded safety and efficacy data for the study. The data will be provided in a standard summary format. The DSMB may be asked to provide recommendations in response to questions posed by DMID.

The DMID DSMB may also be convened for an *ad hoc* meeting, an unplanned meeting that is called for a specific purpose such as when a study halting rule is met. The meeting can be requested by any party with the responsibility of overseeing the trial (such as the PI, ISM, DSMB, DMID, industry collaborator). In the case of an *ad hoc* meeting, the DMID DSMB may request special reports on an as-needed basis.

If the study is discontinued, additional participants will not receive study vaccine. If the study is discontinued, follow-up visits for safety would continue. The DMID DSMB will be notified by DMID when the decision is made to progress to the next cohort.

9.6.2 DMID Independent Safety Monitor (ISM)

The DMID ISM is a local physician with relevant expertise whose primary responsibility is to provide independent safety monitoring in a timely fashion. The ISM will review SAEs in real time and other AEs as needed and provide an independent assessment to the DMID DSMB, when requested. The local study team will identify a DMID ISM and back-up ISM with experience in infectious diseases or gastrointestinal diseases, in close proximity to the participating site, and have the authority to readily access study participant records.

9.6.3 Local DSMB

The icddr,b has its own regulations and standards for safety oversight, which are independent from the DMID DSMB. The Ethical Review Committee (ERC) of icddr,b will arrange for the formation of a local DSMB for this study. The local DSMB is an independent safety oversight body from the DMID DSMB. The membership of the local DSMB consists of at least 2 members from the ERC, as well as 1-2 members from icddr,b who are not the study investigators (~4-5 members); there may also be 1-2 external members (experts) invited to participate in the local DSMB review—thus there are typically 5-6 individuals participating in the local DSMB for a particular study. The study site PI, together with the key investigators, is expected to formally present the study safety data directly to the local DSMB at specified time points during the conduct of the study. The safety data will be generated from web-reports that are updated daily in the electronic data system.

The first anticipated time for a local DSMB review will be prior to initiation of the study and will be followed by meetings for presentation of safety results as soon as it becomes available after the completion of the 7 days follow up visits after the third dose of vaccine for each dosage and route of administration cohort (cohort A-I). Following each local DSMB meeting, a recommendation will be provided to proceed with the next cohort. *Ad hoc* local DSMB meetings will be convened whenever there are any unexpected safety events. Following completion of the study, a final DSMB meeting is held to update the committee on the results.

9.6.4 Independent Protocol Safety Team (IPST)

The IPST will consist of two icddr,b physicians with relevant expertise in infectious and/or gastrointestinal diseases and whose primary responsibility is to provide independent safety monitoring in a timely fashion. The IPST members are located in close proximity to the participating clinical site and have the authority to readily access study participant records. The IPST will review AE and SAEs in real time during the study period and provide an independent assessment of AEs and unexpected events, as needed. The IPST are required to be present when the local icddr,b DSMB meets; they will also be available for the DMID DSMB, when requested. The IPST members may be the same individuals that serve the capacity of DMID ISM and back-up ISM.

10 CLINICAL MONITORING

10.1 Site Monitoring Plan

Site monitoring is conducted to ensure that human subject protection, study procedures, clinical laboratory, study intervention administration, and data collection processes are of high quality and meet sponsor, ICH E6, and other appropriate, regulatory guidelines and that the study is conducted in accordance with the protocol and sponsor standard operating procedures (SOPs). Site visits may be conducted by an authorized representative of DMID or other regulatory agencies to inspect study data, participants' medical records, and CRFs in accordance with ICH guidelines, GCP, and the respective local and national government regulations and guidelines.

The investigator will permit authorized representatives of DMID and the respective local and national health or regulatory authorities to inspect facilities and records relevant to this study if needed. The site PI will provide direct access to all trial-related sites, source data/documents, and reports for the purpose of monitoring and auditing by the sponsor, and inspection by local and regulatory authorities.

DMID-designated clinical monitors will verify that the clinical trial is conducted and data are generated, documented (recorded), and reported in compliance with the protocol, GCP, and the applicable regulatory requirements. Clinical monitoring reports will be submitted to DMID.

A Clinical Monitoring Plan (CMP) will be written and implemented by DMID and its monitoring contractor, ICON.

ICON Clinical Research
212 Church Road
North Wales PA 19454, USA

11 STATISTICAL CONSIDERATIONS

11.1 Study Hypotheses

The study is not designed to test a specific hypothesis for the primary safety objectives, so no formal hypothesis testing is planned. Rather the study is designed to assess the safety and immune responses of a range of doses of dmLT when administered by the oral, SL, or ID route; in volunteers residing in an endemic country for ETEC disease.

11.2 Sample Size Considerations

The sample size for each cohort was chosen based on the number of participants deemed appropriate for a Phase 1 study in which the vaccine has had limited experience by the different routes of administration in humans. We have limited the sample size to at least 11evaluable participants and up to 15 participants for each cohort (each of the nine cohorts A through I).

The DMID DSMB is scheduled to review cumulative data after Cohorts A through F have completed safety data through 7 days post-third dose of vaccine. At which time each cohort is expected to have at least 10 evaluable vaccinees (10 per cohort). Among the 10 vaccinees per dose, the absence of a dose-limiting AE provides for an upper 95% confidence bound of 30.8%. The absence of a dose-limiting AE among 20 vaccinees per route (e.g., combining safety data from Cohort A and B) provides for an upper 95% confidence bound of 16.8%. If at the time of data review, there are no dose-limiting AEs among 60 vaccinees (e.g., combining safety data from Cohorts A through F), this would provide an upper 95% confidence bound of 6.0%.

11.3 Planned Interim Analyses

11.3.1 Safety Review

There is no formal statistical interim analysis based on the safety data. The DMID DSMB will review all safety data through 7 days after the third dose of vaccine from Cohorts A through F, prior to making a recommendation to DMID on proceeding to Cohorts G, H, and I. Data for review will have any outstanding queries resolved but the database will not be locked for this review. The DMID DSMB will advise DMID of its findings. If a halting rule is reached, more frequent meetings may be held. The DMID DSMB will be notified when the decision is made to progress to the next cohort.

A local DSMB will also review safety data and will provide a recommendation with enrollment and vaccination of the next successive dosage and route of administration cohort.

11.3.2 Immunogenicity Review

The immunology data will not be a required element of the data for the DMID DSMB or local DSMB to review. If available, it may be incorporated into the interim safety review. There is no formal statistical interim analysis based on the immunology data.

11.4 Final Analysis Plan

The final analysis will be performed and clinical study report completed when all primary safety endpoint data and all secondary immunogenicity data are available.

11.4.1 Analysis Populations

The Safety Analysis population will include all eligible participants who received at least one dose of study product.

The modified-intention-to-treat (mITT) immunogenicity population includes all eligible participants who received at least one dose of study product and contributed both pre- and at least one post-vaccination samples for immunogenicity testing for which valid results were reported.

The per protocol (PP) immunogenicity population includes all participants in the mITT subset with the following exclusions:

- Data from all available visits for participants found to be ineligible at baseline
- Data from all visits subsequent to major protocol deviations, such as:
    - Second or third vaccination not received
    - Second or third vaccination received out of window
    - Receipt of non-study vaccines during the timeframe prohibited by the protocol
- Data from any visit that occurs substantially out of window

In the case of miss-randomization, participants will be analyzed according to the study product actually received for all analysis populations.

11.4.2 Safety Data

Summaries and analysis of safety data will be presented for the Safety Analysis Population.

Solicited AEs will be summarized by severity for each day after each study vaccination (Days 1-7 post each study vaccination) and as the maximum severity over all 8 days. Additionally, solicited AEs will be analyzed by taking the most severe response over the follow-up period, dichotomizing into a binary variable (none versus mild, moderate, or severe) and using standard techniques, such as exact confidence intervals, to summarize the percentage of participants reporting each symptom, any local symptom, and any systemic symptom. Summaries of solicited AEs will be presented separately for each study vaccination as well as overall study vaccinations by group.

Unsolicited AEs will be coded by Medical Dictionary for Regulatory Activities (MedDRA) for preferred term and system organ class. The number of SAEs is expected to be small in this study and will be reported by a detailed listing showing the event description, MedDRA coding, relevant dates (vaccinations and AEs), severity, relatedness, and outcome for each event. Non-serious unsolicited AEs will be summarized as number and percentage of participants reporting at least one event in each MedDRA preferred term and SOC, cross tabulated by severity and relationship to study product. Additionally, the percentage of participants and exact 95% confidence intervals of AEs in aggregate and by MedDRA categories will be computed. Descriptive statistics for the actual values of clinical laboratory parameters and changes from baseline in clinical laboratory parameters will be presented for all measurements over time. If a participant has repeated laboratory values for a given time point, the value from the last evaluation will be used for analysis.

The number and percentage of participants who terminate the study early (withdraw) or discontinued treatment will be included in the subject disposition summary table. The reason for early termination and discontinuation of treatment will be tabulated if the number of occurrences permits. All early terminations and discontinuations will be listed showing the category (“Early Termination” or “Treatment Discontinuation”), reason, and study day.

11.4.3 Immunogenicity Data

Summaries and analysis of immunogenicity data will be presented for the mITT and PP populations.

Immune responses will be summarized at each time point within each route by treatment, pooling participants who received placebo. Analyses will include number and percentage of participants with responses, as defined by each outcome measure. Descriptive summary statistics

will be provided for all assays and time points. Measurements that are titers or concentrations will be summarized by geometric means and 95% confidence intervals.

12 SOURCE DOCUMENTS AND ACCESS TO SOURCE DATA/DOCUMENTS

The site will maintain appropriate medical and research records for this trial, in compliance with ICH E6, Section 4.9 and regulatory and institutional requirements for the protection of confidentiality of participants. The site will permit authorized representatives of the DMID, its designees, and appropriate regulatory agencies to examine (and when required by applicable law, to copy) clinical study records for the purposes of quality assurance reviews, audits, monitoring and evaluation of the study safety and progress. These representatives will be permitted access to all source data, which include, but are not limited to, hospital records, clinical and office charts, laboratory notes, memoranda, evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate and complete, microfiches, photographic negatives, digital photos, microfilm or magnetic media, x-rays, and participant files and records kept at the pharmacy, at the laboratories, and medico-technical departments involved in the clinical trial. Source data are all information, original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Data collection forms will be derived from the eCRFs and be provided by the Statistical and Data Coordinating Center (SDCC).

13 QUALITY CONTROL AND QUALITY ASSURANCE

Following a written DMID-accepted protocol-specific quality management plan, the investigational site is responsible for conducting routine quality assurance (QA) and quality control (QC) activities to internally monitor study progress and protocol compliance. The PI will provide direct access to all source data/documents, and reports for the purpose of monitoring and auditing by the sponsor, and inspection by local and regulatory authorities. The PI will ensure all study personnel are appropriately trained, and applicable documentations are maintained on site. DMID-designated clinical monitors will verify the clinical trial is conducted and data are generated, documented (recorded), and reported in compliance with the protocol, GCP, and the applicable regulatory requirements. Clinical monitoring reports will be submitted to DMID.

The SDCC will implement quality control procedures beginning with the data entry system and generate data quality control checks that will be run on the database. Any missing data or data anomalies will be communicated to the site for clarification and resolution.

14 ETHICS/PROTECTION OF HUMAN SUBJECTS

Participants in this study will receive no assurance of benefit from their participation. However, participants will receive medical care or referral according to the local standard of care anytime they are sick while enrolled in the study. Those who receive the vaccine, but not those receiving the placebo, might obtain some protection from ETEC diarrhea. If the results of this research study show that the vaccine is safe and likely to be effective in preventing ETEC diarrhea, the vaccine will be further developed and thus there is a societal benefit of this study which may result in preventing ETEC infections.

Nonetheless, the education and training of participants about the rationale, procedures, and risks is critical for the protection of human participants. Prospective participants must demonstrate their persistent interest verbally in the study and compliance with study requirements. There will be multiple opportunities for discussion and questions and answers during the recruiting and screening period and throughout the study period, including time with the investigators.

14.1 Ethical Standard

The investigator will ensure that this study is conducted in full conformity with the principles set forth in The Belmont Report: Ethical Principles and Guidelines for the Protection of Human Subjects of Research of the US National Commission for the Protection of Human Subjects of Biomedical and Behavioral Research (April 18, 1979) and codified in 45 CFR Part 46, 21 CFR 50 and 56 and/or the ICH E6; 62 Federal Regulations 25691 (1997). The study will also be conducted to conform with the ethical standards of Bangladesh, which is to include the Research Review Committee (RRC), Ethical Review Committee (ERC), and the icddr,b DSMB convened at the behest of icddr,b.

14.2 Institutional Review Board

Prior to enrollment of participants into this trial, the approved protocol and informed consent forms, as well as any other participant recruitment materials, or any other materials provided to participants, will be reviewed and approved by the appropriate IRB listed on the institutions FWA. Should amendments to the protocol be required, the amendments will be written by the Sponsor and provided to the site principal investigator for submission to the IRB. Any amendments to the protocol and any materials provided to study participants will be approved by the following IRBs prior to being placed into use:

University of Maryland School of Medicine
Human Research Protections Office
Lexington Building
620 W. Lexington Street, Second Floor
Baltimore, MD 21201
410-706-5037

Icddr,b Ethical Review Committee
Contact-Mr. M.A. Salam Khan
icddr,b, Coordination Committee Secretariat
68, Shaheed Tajuddin Ahmed Sarani, Mohakhali, Dhaka-1212, Bangladesh
Phone +88029886098

14.3 Informed Consent Process

Before any study procedures are performed, participants must sign an informed consent form that complies with the requirements of 21 CFR Part 50 and 45 CFR 46 and the local IRB. The site principal investigator will choose participants in accordance with the eligibility criteria detailed in Section 5.

Participants for this study will be recruited from the Mirpur field site. Study staff will visit nearby households of Mirpur field clinic and have preliminary discussions with adults regarding the study and study procedures. During this process of recruitment, study staff will identify potential participants who are willing to hear more about the study and further invite them to come to our field clinic for a scheduled time of screening. Interested individuals will sign the informed consent forms (ICFs) before any protocol-specific screening procedures are conducted. The consent forms will be utilized by the field staff throughout the recruitment process.

Study personnel may employ this type of recruitment effort prior to obtaining study consent if a patient-specific screening consent is on record or if the IRB has agreed that chart review is allowed without a fully executed screening consent. In cases where there is not a patient-specific screening consent on record, site clinical staff may pre-screen via chart review and refer potential participants to the research staff. Research staff would obtain written consent per the standard informed consent process before conducting protocol-specific screening activities.

Informed consent is an ongoing process that is initiated before the individual's agreeing to participate in the study and continuing throughout the individual's study participation. Extensive discussion of risks and possible benefits of this therapy will be provided to the participants. Consent forms describing in detail the study interventions/products, study procedures, and risks are given to the participant and written documentation of informed consent is required before performing screening activities and enrolling participants on the study.

A delegated consent taker will explain every detail to the potential participant. Consent forms will be translated into Bengali and approved by the local Ethics Board and UMB IRB and the study participant will be asked to read and review the document. Upon reviewing the document, the delegated consent taker or investigator will explain the research study to the participant and answer any questions that may arise. The participants should have the opportunity to discuss the study with their surrogates or consider participation before agreeing to enroll in the study. When the participant has voluntarily accepted all aspects of the study, the consent document will be signed by the participant. The participants will sign the informed consent document before any procedures are done specifically for the study.

A literacy rate of approximately 40% is expected in the Mirpur study area. Bengali is the most commonly spoken language. So there is a chance that a number of illiterate participants will be enrolled. For illiterate participants, an impartial witness will be present during all informed consent discussions between the participant and the delegated consent taker. A person who can only write his/her name but cannot read or write in Bangla will also be considered as illiterate. The Witness must be literate, adult and impartial (i.e., have no relation with study by any means). The Witness will be present during the consent process with the potential participant and will help him/her to understand the study process, study procedures, risk-benefit, etc. The Witness will write the name of study participant on the ICF in the case of an illiterate participant. The Witness will also write his/her name and provide a signature on the appropriate section of ICF. Illiterate participants will need to give a thumbprint for their signature and the Witness will date the form next to the thumbprint. An impartial Witness will be made available to also confirm ongoing consent.

The impartial witness can read, write and understand the ICF, whereas the delegated consent taker (a member of the study team) will explain the ICF and answer any questions to the study participant in the presence of the witness.

DMID will provide the site principal investigator, in writing, any new information that significantly impacts the participants' risk of receiving the investigational product. This new information will be communicated by the site principal investigator to participants who consent to participate in this trial in accordance with IRB requirements. The informed consent document will be updated and participants will be re-consented per IRB requirements, if necessary.

The participants may withdraw consent at any time throughout the course of the trial. The verbal withdrawal of consent will be confirmed by the investigator and reasons for withdrawal if known, and whether the withdrawal is from the entire research study or just the primary interventional component will be documented. A copy of the informed consent document will be given to the participants for their records. The rights and welfare of the participants will be protected by emphasizing that the quality of their medical care will not be adversely affected if they decline to participate in this study. No individual group will be targeted for enrollment.

Every effort will be made to ensure that volunteers do not feel there is any secondary gain for themselves from participation.

14.4 Subject Confidentiality

All information collected about participants will be kept confidential to the extent required by federal, state, and local law.

Participant confidentiality is strictly held in trust by the participating investigators, their staff, the sponsor(s), and their agents. This confidentiality is extended to cover testing of biological samples in addition to the clinical information relating to participating participants. The study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorized third party without prior written approval of the sponsor. All information provided by the Sponsor and all data and information generated by the site as part of the study (other than a participant's medical records) will be kept confidential by the Investigator and other site staff. This information and data will not be used by the Investigator or other site personnel for any purpose other than conducting the study. These restrictions do not apply to: (1) information which becomes publicly available through no fault of the Investigator or site staff; (2) information which it is necessary to disclose in confidence to an IRB solely for the evaluation of the study; (3) information which it is necessary to disclose in order to provide appropriate medical care to a study participant; or (4) study results which may be published as described in Section 16.

The study monitor or other authorized representatives of the sponsor and members of the ethics committee and data and safety monitoring committee may inspect all documents and records required to be maintained by the Investigator, including but not limited to, medical records (office, clinic, or hospital) and pharmacy records for the participants in this study. The FDA or local National Regulatory Authority (Directorate General of Drug Administration, DGDA) may also access participant records. The clinical study site will permit access to such records.

14.5 Future Use of Stored Specimens

Aliquots of unused sera from immunogenicity samples of participants who consented to specimen future use will be stored at DMID CMS for an indefinite period of time, according to the approval policy of the local site's IRB equivalent. Note: the local institution's (icddr,b) Ethical Review Committee (ERC) requires for specimens which are stored outside of Bangladesh to have a limit of 5 years of storage, nonetheless requests to extend the storage for additional 5 year increments is planned indefinitely. Unused Stool collected for future use microbiome

studies and unused residual stool and saliva specimens will be stored at DMID CMS for participants who consented to stool and saliva specimen future use, for at least 2 years. However, if the participant originally consents to future use and subsequently changes his/her decision, any data obtained prior to the withdrawal of consent may still be used for research. The withdrawal of future use consent must be documented; for illiterate participants, this can be completed by an investigator upon confirmation with the study participant. UMB, icddr,b, and investigators in other NIAID approved studies may use the archived specimens for future studies if approved by the clinical site IRB and DMID. Specimens will have a barcode label attached so that any information derived from these research studies will remain confidential. The PBMC will be isolated from the whole blood collected and cryopreserved for memory B cell and T cell assays. No human genetic testing will be done on these specimens. Specimens will not be sold or used for production of any commercial product.

An aliquot of 1-2 grams of stool from each participant as described in the Section 7 and appendix A will be collected and kept frozen at -80°C±15°C until used in the future for microbiome studies.

15 DATA HANDLING AND RECORD KEEPING

The Statistical and Data Coordinating Center at The Emmes Corporation will prepare source documents to match the Advantage eClinical[SM] eCRF screens. These forms are posted to the DMID study web site. The Investigator is responsible for ensuring the accuracy, completeness, legibility, and timeliness of the data reported. All source documents will be completed in a neat, legible manner to ensure accurate interpretation of data. Black or blue ink will be used to ensure clarity of reproduced copies. When making changes or corrections, the original entry will be crossed out with a single line, and the change initialed and dated. No entry will be erased, overwritten, or covered with correction fluid or tape.

Copies of the eCRF will be provided for use as source documents and maintained for recording data for each participant enrolled in the study. These will be maintained in binders for each participant, separated by visit for easy use. The study records will be stored in a locked office or in a password-protected secure database. The study records will be stored indefinitely.

Data reported in the eCRF derived from source documents must be consistent with the source documents or the discrepancies must be explained

15.1 Data Management Responsibilities

Data management will be the responsibility of The Emmes Corporation. All source documents and laboratory reports will be reviewed for accuracy and completeness by the clinical team. AEs will be graded, assessed for severity and causality, and reviewed by the site PI.

Data collection is the responsibility of the clinical trial staff at icddr,b under the supervision of the PI and with oversight from CVD. During the study, the investigators will maintain complete and accurate documentation for the study.

15.2 Data Capture Methods

Clinical data (including AEs, concomitant medications, and solicited events data) will be entered into a 21 CFR Part 11-compliant internet data entry system provided by The Emmes Corporation. The data system includes password protection and internal quality checks, such as automatic range checks, to identify data that appear inconsistent, incomplete, or inaccurate. Clinical data will be entered directly from the data collection forms/source documents.

15.3 Types of Data

Data for this study will include safety, clinical laboratory, and outcome measures (e.g., solicited events, immunogenicity).

15.4 Timing/Reports

Safety review reports will be prepared by Emmes before each DSMB review. After all participants have completed the study, the final report including safety and immunologic data will be prepared.

15.5 Study Records Retention

Study documents will be retained for a minimum of 2 years after the last approval of a marketing application in an ICH region and until there are no pending or contemplated marketing applications in an ICH region or at least 2 years have elapsed since the formal discontinuation of clinical development of the investigational product and the FDA has been notified. These documents will be retained for a longer period, however, if required by local regulations. No records will be destroyed without the written consent of the sponsor. It is the responsibility of the sponsor to inform the investigator when these documents no longer need to be retained. Informed consent forms for future use will be maintained as long as the samples exist.

15.6 Protocol Deviations

A protocol deviation is any noncompliance with the clinical trial protocol, GCP, or protocol-specific MOP requirements. The noncompliance may be either on the part of the participant, the site principal investigator, or the VTEU site personnel. As a result of deviations, corrective actions are to be developed by the site and implemented promptly.

These practices are consistent with ICH E6:

4.5 Compliance with Protocol, Sections 4.5.1, 4.5.2, and 4.5.3

5.1 Quality Assurance and Quality Control, Section 5.1.1

5.20 Noncompliance, Sections 5.20.1, and 5.20.2.

It is the responsibility of the site principal investigator and personnel to use continuous vigilance to identify and report deviations within five working days of identification of the protocol deviation, or within five working days of the scheduled protocol-required activity. All deviations must be promptly reported to DMID, via the DCC's IDES.

All protocol deviations, as defined above, must be addressed in study participant data collection forms. A completed copy of the DMID Protocol Deviation Form must be maintained in the Regulatory File, as well as in the participant's chart. Protocol deviations must be sent to the local IRB/IEC per their guidelines. The site principal investigator and personnel are responsible for knowing and adhering to their IRB requirements.

16 PUBLICATION POLICY

All investigators funded by the National Institutes of Health (NIH) must submit, or have submitted for them, to the National Library of Medicine's (NLM) PubMed Central an electronic version of their final, peer-reviewed manuscripts upon acceptance for publication, to be made publicly available no later than 12 months after the official date of publication. The NIH Public Access Policy ensures the public has access to the published results of NIH funded research. It requires investigators to submit final peer-reviewed journal manuscripts that arise from NIH funds to the digital archive PubMed Central upon acceptance for publication. Further, the policy stipulates that these papers must be accessible to the public on PubMed Central no later than 12 months after publication. All publications resulting from this study shall be reviewed by DMID prior to submittal for publication. NIH contract support shall be acknowledged in all publications. (refer to NOT-OD-08-033; http://grants.nih.gov/grants/guide/notice-files/NOT-OD-08-033.html)

The International Committee of Medical Journal Editors (ICMJE) member journals have adopted a trials-registration policy as a condition for publication. This policy requires that all clinical trials be registered in a public trials registry such as ClinicalTrials.gov, which is sponsored by the National Library of Medicine (NLM). Other biomedical journals are considering adopting similar policies. This trial will be registered in NLM in accordance with the new NLM requirements under the FDAAA. (refer to FDAA 801 requirements; https://www.clinicaltrials.gov/ct2/manage-recs/fdaaa)

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18 SUPPLEMENTS/APPENDICES

APPENDIX A: SCHEDULE OF EVENTS

Oral and Sublingual cohorts												
Study Visit (V)	screen	1	2	3	4	5	6	7	8	9	*Unscheduled visit, upon investigator judgement*	Early Termination[10]
Study Day	-7 to -4	1	8	15	22	29	36	57	114	209		
Window			±1d	±2d	±1d	±2d	±1d	±3d	±1w	±2w		
Study Week		0	1	2	3	4	5	8	16	30		
Informed Consent	X											
Confirm Ongoing Consent		X	X	X	X	X	X	X	X	X		
Eligibility Review	X	X	X	X	X	X	X	X	X			
Collection of demographics	X											
Medical History	X	X	X	X	X	X	X	X	X	X	X	[X]
Concomitant Medications	X	X	X	X	X	X	X	X	X	X	X	[X]
Physical Exam	X	X[2]	X[2]	X[2]	X[2]	X[2]	X[2]	X[2]	X[2]		X	[X]
Vitals Signs	X[3]	X[3]	X[3]	X[3]	X[3]	X[3]	X[3]	X[3]	X[3]	X[3]	X	[X]
Urine pregnancy test[4,9]	X	X		X		X						
VACCINATION		**#1**		**#2**		**#3**						
Pre-vaccination & Post-vaccination assessment		X[5]		X[5]		X[5]						
Memory Aid and thermometer (d)distribute, (c) collect Memory Aid		d	c	d	c	D	c					[c][7]
Confirm date & time of next Visit		X	X	X	X	X	X	X	X			
Adverse Event (AE)/Serious AE review		X	X[6]	X	X[6]	X	X[6]	X[6]	X[1]	X[1]	X[8]	X
Screening stool	X											
Saliva/Oral Fluid for Ab		X	X	X	X	X	X	X			*Also may occur:*	
Stool for ELISA Ab		X	X	X	X	X	X	X				

Stool for future use (Microbiome studies)	X	X	X	X	X	X	X	X			*Stool culture, as when indicated in section 8.2.2; Exam and Photo of local reactogenicity, as when indicated in section 8.1*	
Clinical labs/chemistry	2 mL						2 mL	2 mL				
Blood-Serum for ELISA Ab & Neut.		3 mL	2 mL	2 mL	2 mL	2 mL	2 mL	3 mL	2 mL			
Blood-PBMC for ASC		2 mL	2 mL	2 mL	2 mL	2 mL	2 mL					
Blood-PBMC for ASC w/ homing			2 mL		2 mL		2 mL					
Blood-PBMC for ALS		2 mL	2 mL	2 mL	2 mL	2 mL	2 mL					
Blood-PBMC for Memory B assays		8mL			5 mL		5 mL	5 mL	5 mL	5 mL		
Blood-PBMC for effector T cell assays	13 mL				8mL		7 mL	7 mL	8 mL	10 mL		
Store plasma from PBMC		X	X		X		X	X	X	X		
Total Blood Volume up to ~140 mL/volunteer	15 mL	15 mL	8 mL	6 mL	21 mL	6 mL	22 mL	17 mL	15 mL	15 mL		10 mL

[ ] Make every effort to collect at Early Termination visit. If not collected, this will not result in a protocol deviation.
[1]review for Serious Adverse Events only
[2]Targeted physical examination, if indicated based on review of medical history
[3]Vital signs: collect oral temperature, pulse, and blood pressure
[4]Urine pregnancy test required for all women
[5]Exam at end of 30 min observation period post-vaccination for SL and ID, at end of a 90 min observation period for Oral
[6]Review to include assessments of: 1) injection site necrosis within 7 days of vaccination, 2) urticaria, laryngospasm, bronchospasm, or anaphylaxis determined to be related to vaccine and within 7 days of vaccination, 3) facial nerve neuropathy, confirmed by an investigator and determined to be related to vaccine.
[7]Assess reactogenicity and review memory aid if early termination occurs within 7 days of any vaccination
[8] AE assessment to include (as applicable): 1) Assess reactogenicity (if within 7 days of last vaccination), unsolicited non-serious AE information (if within 28 days of last vaccination), or any SAE information (anytime within study) 2) Upon judgement of the clinical investigator, clinical laboratory assessments may be performed to evaluate an AE and medications (e.g analgesics) may be recommended to treat an AE.
[9]Visits 1-7: Provide instructions to women participants to avoid pregnancy through the 28 days from receipt of the last dose of vaccine.
[10]Early Termination Visit is to include: assess reactogenicity and review memory aid if early termination occurs within 7 days of any vaccination; assess for SAE during all early termination visits; obtain serum samples for laboratory safety assays if visit occurs <28 days after third vaccination.

Intradermal cohorts												
Study Visit (V)	screen	1	2	3	4	5	6	7	8	9	*Unscheduled visit, upon investigator judgement*	Early Termination[11]
Study Day	-7 to -4	1	8	22	29	43	50	71	128	223		
Window			±1d	±2d	±1d	±2d	±1d	±3d	±1w	±2w		
Study Week		0	1	3	4	6	7	10	18	32		
Informed Consent	X											
Confirm Ongoing Consent		X	X	X	X	X	X	X	X	X		
Eligibility Review	X	X	X	X	X	X	X	X	X			
Collection of demographics	X											
Medical History	X	X	X	X	X	X	X	X	X	X	X	[X]
Concomitant Medications	X	X	X	X	X	X	X	X	X	X	X	[X]
Physical Exam	X	X[2]	X[2]	X[2]	X[2]	X[2]	X[2]	X[2]	X[2]		X	[X]
Vitals Signs	X[3]	X[3]	X[3]	X[3]	X[3]	X[3]	X[3]	X[3]	X[3]	X[3]	X	[X]
Urine pregnancy test[4,10]	X	X		X		X						
VACCINATION		**#1**		**#2**		**#3**						
Pre-vaccination & Post-vaccination assessment		X[6]		X[6]		X[6]						
Memory Aid and thermometer (d)distribute, (c) collect Memory Aid		d	c	d	c	d	c					[c][8]
Confirm date & time of next Visit		X	X	X	X	X	X	X	X			
Adverse Event (AE) / Serious AE review		X	X[5,7]	X	X[5,7]	X	X[5,7]	X[7]	X[1]	X[1]	X[5,9]	X
Screening stool	X											
Saliva/Oral Fluid for Ab		X	X	X	X	X	X	X			*Also may occur: Stool culture, as when indicated in section 8.2.2;*	
Stool for ELISA Ab		X	X	X	X	X	X	X				
Stool for future use (Microbiome studies)	X	X	X	X	X	X	X	X				
Clinical labs/chemistry	2 mL						2 mL	2 mL				
Blood-Serum for ELISA Ab & Neut.		3 mL	2 mL	2 mL	2 mL	2 mL	2 mL	3 mL	2 mL			
Blood-PBMC for ASC		2 mL	2 mL	2 mL	2 mL	2 mL	2 mL					

Blood-PBMC for ASC w/ homing			2 mL		2 mL		2 mL				*Exam and Photo of local reactogenicity, as when indicated in section 8.1*	
Blood-PBMC for ALS		2 mL	2 mL	2 mL	2 mL	2 mL	2 mL					
Blood-PBMC for Memory B assays		8mL			5 mL		5 mL	5 mL	5 mL	5 mL		
Blood-PBMC for effector T cell assays	13 mL				8 mL		7 mL	7 mL	8 mL	10 mL		
Store plasma from PBMC		X	X		X		X	X	X	X		
Total Blood Volume up to ~140 mL/volunteer	15 mL	15 mL	8 mL	6 mL	21 mL	6 mL	22 mL	17 mL	15 mL	15 mL		10 mL

[ ] Make every effort to collect at Early Termination visit. If not collected, this will not result in a protocol deviation.
[1]review for Serious Adverse Events only
[2]Targeted physical examination, if indicated based on review of medical history
[3]Vital signs: collect oral temperature, pulse, and blood pressure
[4]Urine pregnancy test required for all women
[5]For grade 3 local site reactions, a photo should be taken on a daily basis until the injection site reaction is deemed stable or returns back to a grade 2 or lower severity, if the event occurs within 7 days of an intradermal dose administration
[6]Exam at end of 30 min observation period post-vaccination
[7]Review to include assessments of: 1) injection site necrosis within 7 days of vaccination, 2) urticaria, laryngospasm, bronchospasm, or anaphylaxis determined to be related to vaccine and within 7 days of vaccination, 3) facial nerve neuropathy, confirmed by an investigator and determined to be related to vaccine.
[8] Assess reactogenicity and review memory aid if early termination occurs within 7 days of any vaccination
[9]AE assessment to include (as applicable): 1) Assess reactogenicity (if within 7 days of last vaccination), unsolicited non-serious AE information (if within 28 days of last vaccination), or any SAE information (anytime within study) 2) Upon judgement of the clinical investigator, clinical laboratory assessments may be performed to evaluate an AE and medications (e.g analgesics) may be recommended to treat an AE.
[10]Visits 1-7: Provide instructions to women participants to avoid pregnancy through the 28 days from receipt of the last dose of vaccine.
[11]Early Termination Visit is to include: assess reactogenicity and review memory aid if early termination occurs within 7 days of any vaccination; assess for SAE during all early termination visits; obtain serum samples for laboratory safety assays if visit occurs <28 days after third vaccination.

APPENDIX B: ACCEPTABLE SCREENING LAB VALUES

Test	Reference Range	Acceptable Range	Units
White Blood Cell count (WBC)	4.0-11.0	4-11	cells $x10^9$ /Liter
Absolute Neutrophil Count (ANC)	2.0-7.5	2-7.5	cells $x10^9$ /Liter
Hemoglobin (Hg) – Female	11.5-16.5	9.5-16.5	gm/dL
Hemoglobin (Hg) – Male	12.5-17.5	11.0-17.5	gm/dL
Platelets	150-450	150-450	cells x 10^9 /Liter
Creatinine, Female	44-97	≤97	µmol/Liter
Creatinine, Male	53-106	≤106	µmol/Liter
Albumin	34.0-50.0	≥34	g/L
Alanine Aminotransferase (ALT), Female	0.01 - 31.0	≤31	U/L
Alanine Aminotransferase (ALT), Male	0.01 - 41.0	≤41	U/L
Hepatitis B surface antigen (HBsAg)	Non Reactive	Non Reactive	n/a
HCV antibody	Non Reactive	Non Reactive	n/a
Total Bilirubin	5.0 - 21.0	5.0 - 21.0	µmol/Liter

APPENDIX C: LABORATORY AND CLINICAL TOXICITY GRADING SCALES

Solicited Lab AEs	Grade 1 – Mild	Grade 2 – Moderate	Grade 3 - Severe
WBC, Decreased (cells x10^9 /Liter)	2.0 – 3.9	1.5 – 1.9	≤1.4
WBC, Increased (cells x10^9 /Liter)	11.1 – 15.0	15.1 – 20.0	≥20.1
ANC, Decreased (cells x10^9 /Liter)	1.0 – 1.9	0.75 – 0.99	≤0.74
Hemoglobin female (gm/dL)	9.0- 9.4	8.0 - 8.9	6.5 – 7.9
Hemoglobin, male (gm/dL)	10.0 - 10.9	9.0 – 9.9	7.0 – 8.9
Platelets (cells x 10^9 /Liter)	100 – 149	50 – 99	≤49
Creatinine (µmol/Liter)	1.1 – 1.3 x ULN	>1.3 – 1.8 x ULN	>1.8 – 3.4 x ULN
ALT (SGPT) (U/L)	1.25 – 2.5 x ULN	>2.5 – 5.0 x ULN	>5.0 – 10.0 x ULN
Albumin (g/dL)	2.8 - 3.1	2.5 - 2.7	< 2.5
Total Bilirubin	1.1 to <1.6 x ULN	1.6 to <2.6 x ULN	≥2.6 x ULN

ULN = upper limit of normal, from the laboratory reference range

Clinical Sign	Normal	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)
Temperature, °C	35-37.9	38.0 to 38.4	38.5 to 38.9	≥39.0
Tachycardia, bpm	≤100	101 to 115	116 to 130	>130
Bradycardia, bpm	≥50	45 to 49	40 to 44	<40
Hypertension, systolic, mmHg	≤140	141 to 150	151 to 155	>155

Clinical Sign	Normal	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)
Hypotension, systolic, mmHg	≥90	85-89	80-84	<80
Hypertension, diastolic, mmHg	≤90	91-95	96-100	>100
Hypotension, diastolic, mmHg	≥60	45-59	35-44	<35
Feverishness (Chills/Shivering/Sweating)	none	Mild, but no interference with function	Some interference with daily activity	Significant interference, prevents daily activity
Fatigue (Tiredness)	Normal activity reduced slightly	Normal activity decreased 25-50%	Unable to perform daily activity	Urgent care visit or hospitalization
Malaise (General Unwell Feeling)	No interference with daily activity	Some interference with daily activity	Significant interference with daily activity	Urgent care visit or hospitalization
Myalgia (Body/Muscle Aches)	Mild, but no interference with function	Moderate, interferes with function, but not with daily activity	Severe, interferes with daily activity	Severe and disabling, unable to perform daily activity
Headache	Mild and brief or easily tolerated	Moderate with limited effect to daily activity	Severe and prevents daily activity	Urgent care visit or hospitalization
Allergic reaction	Pruritus, no rash	Localized urticaria	Generalized urticarial, angioedema	Anaphylaxis
Diarrhea	Normal or 1-2 loose stool per 24 hr	3 loose stools per 24 hr	4-7 loose stools per 24 hr	8 or more loose/watery stools per 24 hr or requires outpatient IV hydration, ED visit or hospitalization for diarrhea

Clinical Sign	Normal	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)
Nausea	none	Mild, but no interference with activity or intake	Moderate, interferes with activity or intake	Severe, unable to perform activity or no intake
Vomiting	none	1-2 episodes per 24 hr.	3-4 episodes per 24 hr.	≥5 episodes per 24 hr.
Abdominal Discomfort	No interference with daily activity	Some interference with daily activity	Significant interference with daily activity	Urgent care visit or hospitalization
Irritation of Oral Cavity (Mouth Pain/Sores)	none	Mild or transient, no limitation of oral intake	Moderate, slight interference with oral intake	Severe, unable to tolerate oral intake
Facial Nerve Disturbance	none	Mild or transient, no limitation	Moderate disturbance but no overt facial nerve palsy	Severe, obvious facial nerve palsy
Injection Site Pain	none	Aware of pain but no interference with activity	Slight interference with activity, may require analgesic	Pain that prevents daily activity
Injection Site Redness	none	Less than 5x5 cm	Greater than 5x5 cm but less than 9x9 cm	Greater than 9x9 cm
Injection Site Swelling	none	Less than 5x5 cm	Greater than 5x5 cm but less than 9x9 cm	Greater than 9x9 cm or ulceration or necrosis
Injection Site Bruising (Ecchymosis)	none	Less than 5x5 cm	Greater than 5x5 cm but less than 9x9 cm	Greater than 9x9 cm
Injection Site Itching	none	Aware of sensation but no	Slight interference with activity,	Itching that prevents daily activity

Clinical Sign	Normal	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)
		interference with activity	may require medication	
Injection Site Induration (limited to the dermal layer)	none	Less than 5x5 cm	Greater than 5x5 cm but less than 9x9 cm	Greater than 9x9 cm
Injection Site Hypo/Hyperpigmentation	none	Less than 1x1 cm	Greater than 1x1 cm but less than 5x5 cm	Greater than 5x5 cm
Injection Site Vesicles	none	≤5 vesicles and localized to injection site	>5 vesicles and localized to injection site	Any number of vesicles which are generalized in distribution
Injection Site Hardened Mass (deeper than dermal layer)	none	Less than 1x1 cm	Greater than 1x1 cm but less than 5x5 cm	Greater than 5x5 cm
Other illness	No interference with daily activity	Some interference with daily activity	Significant interference with daily activity	Urgent care visit or hospitalization

bpm = beats per minute