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VLA1701-201

Statistical Analysis Plan

Final, 1.0, dated 16-Aug-2018

ASSIGN DMB

Statistical Analysis Plan

Interim and Final Analysis

RANDOMIZED DOUBLE-BLINDED PILOT STUDY CONFIRMING A HUMAN CHALLENGE MODEL USING LSN03-016011/A EXPRESSING LT AND CS17 AND INVESTIGATING THE SAFETY OF VLA 1701 (AN INVESTIGATIONAL ORAL CHOLERA AND ETEC VACCINE)

Protocol: VLA1701-201

Confidential

Sponsor: Valneva Austria GmbH

Adapted from:
STAT03_A Statistical Analysis Plan
Version 6.0, Effective Date 16-Feb-2018

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FINAL 1.0	16-Aug-2018	First version

Approval

[redacted]

List of Abbreviations

AE	Adverse event
ALS	Antibody Lymphocyte Supernatant
ALT	Alanine aminotransferase
ATC	Anatomical therapeutic chemical
BMI	Body Mass Index
BUN	Blood urea nitrogen
C	Celsius
CEC	Clinical Endpoint Committee / Independent Outcome Adjudication Committee
CFU	Colony Forming Unit
CI	Confidence Interval
CSR	Clinical Study Report
cm	Centimeter
ECG	Electrocardiogram
eCRF	Electronic case report form
e.g.	For example
ETEC	Enterotoxigenic E coli
F	Fahrenheit
g	Gramm
GCP	Good Clinical Practice
HBsAG	Hepatitis B surface antigen
HIV	Human immunodeficiency virus
IBS	Irritable Bowel Syndrome
ICF	Informed consent form
i.e.	Id est/ that is
ICH	International Conference on Harmonization
IgA	Immunoglobulin A
IgG	Immunoglobulin G
IV	Intravenous
IVF	Intravenous Fluids
LT	Labile toxin
µg	Microgram
mL	Milliliter
PCR	Polymerase chain reaction
PBMC	Peripheral blood mononuclear cell
PD	Protocol deviation
PT	Preferred Term
SAE	Serious adverse event
SD	Standard deviation

SOC	System Organ Class
SOP	Standard operating procedure
ST	Stable toxin
TLF	Tables , Listings and Figures
WBC	White blood count
WHO	World Health Organization

1. OVERVIEW

1.1 Study Design

This is a single-center, double-blind, placebo-controlled, Phase II vaccination and challenge study designed to confirm a human challenge model with E. coli strain LSN03-016011/A (LT+, ST-, CS17), as well as collect expanded safety and immunogenicity data. The study will be carried out in two phases:

Vaccination phase: up to 34 subjects will be randomized 1:1 to receive 2 doses of either VLA1701 or placebo orally. The doses will be given 7 days apart and subjects will be followed as an outpatient for safety.

Challenge Phase: 30 Subjects, out of the 34 subjects, will be challenged (see section 9.4 of the Clinical Study Protocol). In the morning, after approximately 90 minutes of fasting, 30 subjects will ingest 120 ml of USP sodium bicarbonate solution (13.35 g/liter) followed in 1 minute by another 30-ml bicarbonate solution containing the challenge strain. Subjects continue to fast for 90 minutes following challenge.

After challenge, subjects will be monitored for diarrhea and other signs/symptoms of enteric illness by daily medical checks, vital sign determinations, grading and weighing of all stools.

Five days after challenge, or sooner if subjects meet early treatment criteria, subjects will be treated with antibiotics. Subjects will be discharged after at least 2 doses of antibiotic treatment, clinical symptoms are resolved or resolving and the subject has produced two stool samples that were negative for LSN03-016011/A by microbiological culture. All subjects will be followed up with an in-person visit at Day 44 after vaccination (i.e., 28 days after challenge); a telephone call to check for any serious medical conditions, new onset of chronic illnesses and functional bowel disorders will be scheduled approximately 6 months after their first vaccination.

The overall study design is displayed in Figure 1.

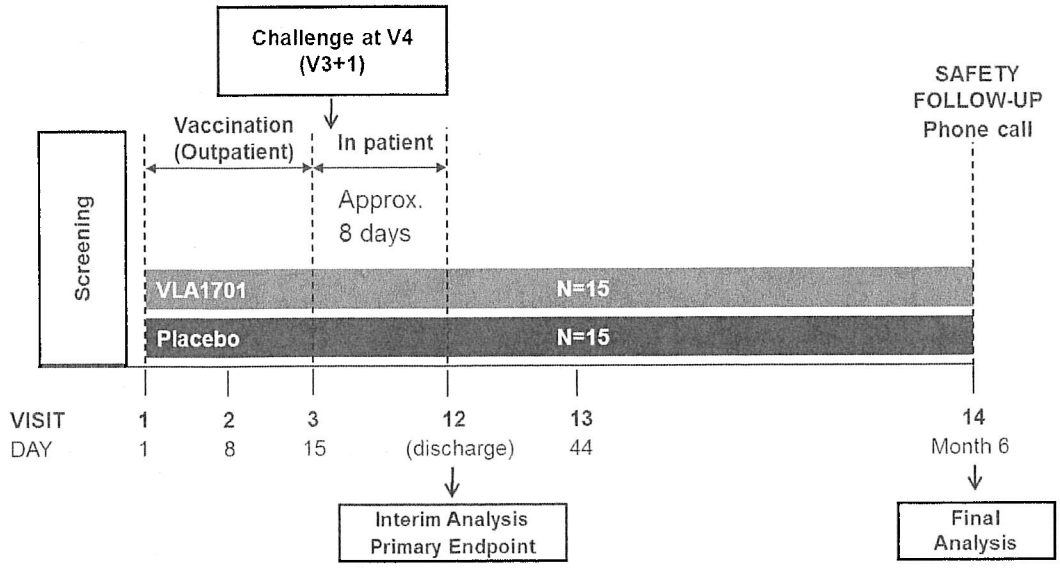

Figure 1: Overall Clinical Phase 2 Study Design

1.2 Study Objectives

1.2.1 Primary Objective

The primary objective of this study is to evaluate the number of subjects with moderate to severe diarrhea after challenge with ETEC strain LSN03-016011/ A.

1.2.2 Secondary Objectives

- To assess the scale of disease severity following Human Challenge with ETEC strain LSN03-016011/ A as described by Porter, et al; An Evidenced-Based Scale of Disease Severity following Human Challenge with Enteroxigenic Escherichia coli; Plos one, 2016.
- To assess the safety of VLA1701 in a healthy adult population aged 18 to <50 years.

1.3 Endpoints

1.3.1 Primary Endpoint

- Percentage of subjects with severe to moderate diarrhea within 120 hours of challenge with ETEC strain LSN03-016011/A.

1.3.2 Secondary Endpoints

Severity of disease induced after challenge with ETEC strain LSN03-016011/ A

- Calculation of disease severity score after challenge with ETEC strain LSN03-016011/ A

Safety of VLA-1701

- Percentage of subjects with solicited adverse events within 7 days after each vaccination.
- Percentage of subjects with any adverse events (AEs) observed up to Visit 4 (before Challenge) and during the entire study period (including clinically significant laboratory parameter changes).
- Percentage of subjects with serious adverse events (SAEs) observed up to Visit 4 (before Challenge) and during the entire study period.
- Percentage of subjects with any Investigational Medicinal Product (IMP)-related AEs observed up to Visit 4 (before Challenge) and during the entire study period (including clinically significant laboratory parameter changes).
- Percentage of subjects with IMP-related SAEs observed up to Visit 4 (before Challenge) and during the entire study period.

1.3.3 Exploratory Endpoints

Disease induced after challenge with ETEC strain LSN03-016011/ A

- To quantify the number and quality of loose stools and grade the intensity of symptoms occurring after challenge.
- Number and percentage of subjects with diarrhea of any intensity.

- Number and percentage of subjects with diarrhea of any intensity, based on evaluation of stool volume only.
- Time to onset of diarrhea from the time of receiving the ETEC challenge (incubation period).
- Time, in hours, from the first diarrheal stool to the last diarrheal stool (duration of diarrhea).
- Mean total weight of grade 3-5 stools passed per subject.
- Mean number of grade 3-5 stools per subject.
- Total mass/volume of diarrheal stools.
- Maximum 24-hour stool output (volume and frequency).
- Number and percentage of subjects requiring early intervention with antibiotic therapy due to the severity of diarrhea.
- Number and percentage of subjects with fever, nausea, bloating, vomiting, myalgia, arthralgia, abdominal pain, abdominal cramping, malaise, headache, light headedness.
- Number of colony forming units (cfu) of the challenge strain per gram of stool on days 2 and 4 after challenge (V6 and V8).
- Number and percentage of subjects requiring IV fluids.
- Number and percentage of subjects developing moderate to severe diarrhea by ABO blood type.

Immunogenicity of VLA-1701

- Systemic immune responses after two doses of VLA1701 at 7 days post vaccination.
- Mucosal immune responses after two doses of VLA1701 at 7 days post vaccination.
- Systemic immune responses after challenge with ETEC strain LSN03-016011/A at 7 days and 1 month after challenge.
- Mucosal immune responses up to 1 month after challenge with ETEC strain LSN03-016011/A at 7 days and 1 month after challenge.

1.4 Sample Size Calculation

Up to 34 subjects will be randomized 1:1 to receive either VLA1701 or placebo, 30 subjects will be challenged.

There was no formal sample size calculation for this pilot study. Sample size was planned considering site capabilities and reference of studies previously described for challenge studies. The primary objective is to gain information on disease induced by the challenge strain in the placebo group in order to confirm published characteristics of the challenge strain and to appropriately design future studies based on the findings of this pilot study (e.g. if 7 or more subjects in Placebo group develop diarrhea, a diarrhea rate induced by the challenge strain of 70% or above cannot be excluded with one-sided 95% Confidence Interval (CI)).

1.5 Flowchart

Visit	V0[a]	V1	V2	V3	V4	V5	V6	V7	V8	V9, 10 [a)]	V11	V12	V13	V14
Timing	Day -60 to -1	Day 1	Day 8	Day 15	V3 + 1	V4 + 1	V4 + 2	V4 + 3	V4 + 4	V4 + 5, +6	V4 + 7	V4 + 8	V4 + 28 days	Month 6
Time windows	-60 to -1	0	0	0 to +3	0 to +3	0	0	0	0	0	0	0	-2 to +2	-14 to -1
Visit type	Screening	Outpatient visit, First vaccination	Outpatient visit, Second vaccination	In-patient	In-patient	In-patient	In-patient	In-patient	In-patient	In-patient	In-patient/ Outpatient	Discharge	Out-patient visit	Safety Follow-up Phone call
Informed Consent	X													
Demography [b)]	X													
Eligibility criteria	X	X	X	X	X									
Pregnancy test	X (serum or urine)	X (urine)	X (urine)	X (serum, urine)									X (urine)	
Medical history	X	X												
Drug screen	X													
Hematology/ Serum Chemistry	X			X							X			
Admission to inpatient unit/discharge				X	X	X	X	X	X	X	X	X		
Physical exam	X			X										
Focused physical exam		X	X		X	X	X	X	X	X	X	X	(X)	
Vital signs	X	X	X	X	X	X	X	X	X	X	X	X	X	
ECG				X										
Symptoms/AEs			X	X	X	X	X	X	X	X	X	X	X	X

Visit	V0	V1	V2	V3	V4	V5	V6	V7	V8	V9, 10 [a]	V11	V12	V13	V14
Timing	Day -60 to -1	Day 1	Day 8	Day 15	V3 + 1	V4 + 1	V4 + 2	V4 + 3	V4 + 4	V4 + 5, +6	V4 + 7	V4 + 8	V4 + 28 days	Month 6
Memory Card		dispense	collect/ dispense	collect										
IBS Survey	X													X
Concomitant therapy		X	X	X	X	X	X	X	X	X	X	X	X	
Randomization		X												
Vaccination		X	X											
Bacterial inoculation					X									
Antibiotic administration						(X) [c]	(X) [c]	(X) [c]	(X) [c]	X	X			
Stool collection [e]	X	(X) [d]		X	X	X	X	X	X	X	X	X	X	
Grading and weighing					X	X	X	X	X	X	X	X		
Stool culture					X	X	X	X	X	X	X	X		
CFU quantification in stool							X		X					
Fecal IgA	X	X [d]		X							X		X	
Serum IgG		X		X							X		X	
Serum IgA		X		X							X		X	
ALS		X		X							X		X	

a) optional visits
b) include date of birth, height, weight, BMI, gender and race
c) antibiotic treatment will be started according to criteria described in the CSP
d) if not collected at Visit 0
e) Stool samples will be collected for assays as specified in the laboratory study of event schedule and as per written study specific procedures. A subset of these samples, during high shedding points, will be reserved for the later validation and development of bacteriological assays for shedding of ETEC and other organisms. Additionally, stool samples will be obtained to assess for exploratory endpoints to include microbiome, PCR, other immunology assays and transcriptomics.

2. GENERAL CONSIDERATIONS

2.1 Conduct of Analysis

An interim analysis including the primary endpoint analysis will be performed after all subjects have gone through challenge and have been released from the inpatient facility (Visit 12). Only demographic details, the primary endpoint and the number of subjects with diarrhea of any intensity by severity will be analyzed in the interim analysis.

A final analysis will be performed once the last subject has completed the study, i.e. Month 6 (Visit 14) and a final Clinical Study Report (CSR) will be compiled.

The primary endpoint and subjects with diarrhea of any intensity will be assessed by the Independent Outcome Adjudication Committee / Clinical Endpoint Committee (CEC), see protocol Section 17.8. This assessment will be performed prior to unblinding of sponsor personnel and statisticians. Secondary and exploratory endpoints that are affected by the CEC's assessment may be re-calculated or re-determined for specific subjects based on the CEC's recommendations. Any such re-calculations and re-assessments of secondary or exploratory endpoints that are not in the scope of the Interim Analysis may occur after unblinding of sponsor personnel and statisticians for Interim Analysis, but the CEC itself will remain blinded until the Final Analysis.

The basis for discussion for the CEC will be data summarized per subject that will be provided in advance. Details on the scope and conduct of the CEC are described in the Adjudication Committee Charter (Version 1.0, July 2018).

2.2 Statistical Software and Quality Control

All statistical analyses will be performed using SAS® version 9.3 or higher. Tables, figures and data listings will be generated in Microsoft® Word® as well as PDF® format.

Quality control of SAS® programs will include independent re-programming according to SOP SAS04 and a review of the whole process of result generation:

- Review of all analysis SAS® programs
- Review of SAS® log for errors, warnings and other notes that could indicate mistakes in the programs
- Review of all tables, listings and figures for completeness and correctness

2.3 Applicable Standard Operating Procedures

The applicable Standard Operating Procedures (SOPs) of Assign DMB for this study are:

STAT01 Statistical Analysis File
STAT03 Statistical Analysis Plan
STAT04 Interim Analysis
STAT05 Randomization and Unblinding
STAT06 Data Review Meeting
STAT07 Report Writing

SAS01 SAS General Principles

SAS04 Handling of Statistical Analyses

2.4 Blinding, Randomization and Challenge Subject Selection

Subjects were randomized in a 1:1 ratio to either receive VLA1701 or placebo. Thirty eligible subjects were challenged. Details on randomization and challenge subject selection processes were pre-defined in a separate document (Randomization and challenge subject selection, FINAL 2.0, 05-Apr-2018).

Only pharmacy staff responsible for IMP preparation, the Lead Biostatistician that prepared the randomization list, Emmes personnel responsible for randomization processes and a designated Emmes Clinical Research Associate (CRA) are unblinded.

2.5 Descriptive Analyses

Descriptive analyses of continuous variables (summary statistics) will be described with the number of non-missing observations, arithmetic mean, standard deviation (±SD), median, quartiles (Q1 and Q3) and range (minimum and maximum). For immunogenicity summaries, the geometric mean will be included.

Categorical variables (frequency statistics) will be described with the number of non-missing observations and percentages (%). Percentages will be calculated within each stratum on the total number of non-missing observations, if not stated otherwise.

2.6 Inferential Analyses

The primary endpoint (moderate/severe diarrhea as assessed by the Independent Outcome Adjudication Committee) will be compared between the two treatment groups using Fisher's exact test and rates will be accompanied by two-sided 95%-confidence intervals (according to Altman). This will be applied also for diarrhea of any intensity and all other categorical efficacy parameters.

All continuous efficacy parameters (excl. the disease severity score) will be compared between treatment groups with the Wilcoxon-Mann-Whitney test.

The disease score as well as the score components will be compared between groups by using the Cochran-Armitage test of trends (the trend being the natural order of the ordinal score). As secondary comparison, a Student's t-test will be applied on the scores.

Geometric means of immunogenicity endpoints will be accompanied by a 95% confidence interval.

All adverse event rates (excl. rates on the SOC and PT level) will be compared between the two groups using Fisher's exact test. Adverse event rates will be accompanied by two-sided 95% confidence intervals (according to Altman).

All tests will be performed under a 2-sided significance level of 0.05.

2.7 Center and Country Effect

Not applicable. This is a single-center study.

2.8 Handling Missing Data

Generally, missing values of immunogenicity and efficacy variables will not be imputed, and the analysis will be limited to observed values. For missing data in AE evaluation (e.g. missing information if serious, medically attended, about severity or causality) a worst case approach will be applied. In case of missing assignment to solicited or unsolicited, this AE will neither be counted in tables for solicited AEs nor in tables for unsolicited AEs but in tables for all AEs.

2.9 Protocol Deviations

Protocol deviations will not lead to exclusion from any analysis population. Deviations reported in the eCRF will be reviewed in the Data Review Meeting (DRM) prior to database closure, listed in the Final Analysis and described in the CSR. Categories for PDs to be used for rate of subjects with deviations will be established during the PD review in the DRM.

2.10 Medical Coding

Adverse events and medical history will be coded using MedDRA. Concomitant medications will be coded using WHO Drug Reference List and Anatomical Therapeutic Chemical (ATC) Classification System.

2.11 Analysis Populations

2.11.1 Safety Population

The safety population contains all subjects who entered into the study and received at least one vaccination. Subjects will be analyzed as treated: Subjects that received at least one VLA1701 vaccination are analyzed in the VLA1701 group. Subjects that only received placebo vaccinations are analyzed in the placebo group.

2.11.2 Challenge Population

The challenge population will be defined as subjects who received two vaccinations and the challenge dose.
The primary analysis will be done in the challenge population. Subjects will be analyzed according to the treatment group they had been allocated to, rather than by the actual treatment they received.
Since this is a pilot study and the main objective is to gain information on disease induced by the challenge strain in placebo subjects, if any subject receives the wrong product then a sensitivity analysis will be performed where subjects will be analyzed as treated.

2.12 Subject Data Listings

All treated subjects will be included in listings if not stated otherwise. Data listings will include the subject number as identifier (and parameter and/or visit if available) and will be sorted by treatment group and subject ID (and

parameter and/or visit if available). A column that indicates if a subject is in the challenge population will be shown in all listings.

2.13 Changes in the Conduct of the Study or Planned Analysis

The following changes as compared to the Clinical Study Protocol (CSP) are planned in this SAP:

- The wording in the CSP for analysis populations indicated that only subjects receiving VLA1701 will be included in analysis populations. This is an obvious inaccuracy since the goal of the study is to compare the subjects receiving placebo and the subjects receiving VLA1701. The wording in the SAP was made clearer (Section 2.11).
- A Student's t-test was planned in the CSP for the comparison of disease severity scores between groups. As the test was judged not optimal for the analysis of an ordinal non-continuous variable, a Chochran-Armitage test of trend was introduced as primary test for this endpoint. The t-test will also be performed as secondary comparison.
- In Section 8.8.2.1 of the CSP, the duration of diarrhea is defined as time from first to last diarrheal stool. During SAP generation it was decided that this way of calculation does not appropriately consider time spans with normal stool output. The calculation of diarrhea duration was re-defined as described in Section 5.2.2 of this SAP.

3. OVERALL STUDY INFORMATION

The following information will be analyzed descriptively and corresponding details on the subject level will be provided in data listings:

- Number of subjects per analysis population
- Subjects per vaccination
- Challenged Subjects
- Subject specific protocol deviations and deviations on the study level (will only be listed)
- Screening failures
- Study visits
- Study Status (incl. drop-outs and reason)
- Discontinuation of Treatment (incl. reason)

Specifications on TLFs are provided in Section 8.

4. BASELINE EVALUATION

Baseline data are presented for the Safety and Challenge Population.

4.1 Data Points and Analyses

The following information will be analyzed descriptively and corresponding details on the subject level will be provided in data listings:

- Demographics (age, sex, race)

- Weight, Height
- Serology (will only be listed)
- Drug Screening (will only be listed)
- Birth Control details (will only be listed)
- Pregnancy test (will only be listed)
- Medical History
- Prior/concomitant Medications
- Physical examination (will only be listed)
- Vital signs at screening (temperature, blood pressure, pulse)

Specifications on TLFs are provided in Section 8.

4.2 Derivations and Definitions

- Body weight will be converted from pounds to kilogram (kg) (weight [kg] = weight [lbs] × 0.45359237.
- Body height will be converted from inch (in) to centimeter (cm) (height [cm] = height [in] × 2.54).
- Body temperature will be converted from degree Fahrenheit (F) to degree Celsius (C) (temperature [°C]=(temperature [°F] – 32) × 5/9).
- Medications stopped clearly prior (<) to Day 0 (Visit 1) will be considered prior medications, all other medications are considered to be concomitant. Medications with a missing or incomplete end date where it cannot clearly be decided if the end date was before or after Day 0 (Visit 1) or if "yes" is ticked for "ongoing" will be considered concomitant.

5. EFFICACY ANALYSIS

All tables and listings will be provided for the challenge population.

5.1 Primary Efficacy Endpoint

The primary endpoint of this study is the occurrence of severe to moderate diarrhea within 120 hours of challenge with ETEC strain LSN03 016011/A.

The CEC will assess the primary endpoint for every subject based on the subject's stool log data. In particular, the number and volume of stools of grade 3-5 (grade 3, viscous opaque liquid or semiliquid which assumed the shape of the container; grade 4, watery opaque liquid; and grade 5, clear watery or mucoid liquid) will be used.

The primary endpoint will be analyzed in the challenge population and will compare the two treatment groups as described in Section 2.6.

5.2 Secondary and Exploratory Efficacy Endpoints

5.2.1 Data Points and Analyses

Summary tables and data listings will be provided for the following:

- Emesis log (will only be listed)
- Disease severity score (including statistical test as described in Section 2.6)
- Total volume of loose stools
- Total volume of loose stools by grade
- Subjects with diarrhea of any intensity and by intensity (subjects with any loose stool).
- Subjects with diarrhea of any intensity and by intensity, based on evaluation of stool volume only.
- Time to onset of diarrhea from the time of receiving the ETEC challenge (incubation period).
- Subjects with moderate or severe diarrhea based on stool volume only (>400g in 24 h period)
- Time to onset of moderate or severe diarrhea from the time of receiving the ETEC challenge (incubation period).
- Time to onset of moderate or severe diarrhea based on stool volume only
- Time from the first diarrheal stool to the last diarrheal stool (duration of diarrhea).
- Mean total weight of grade 3-5 stools.
- Number of grade 3-5 stools per subject.
- Total volume of diarrheal stools.
- Maximum 24-hour stool volume and frequency for stool of any grade
- Maximum 24-hour stool volume and frequency of grade 3-5 stools
- Antibiotic therapy: frequency tables for information on the Antibiotic Administration Record of the CRF; listing that shows the Antibiotic Administration Record together with antibiotics reported in the Concomitant Medication section of the CRF (ATC codes J01EE, J01MA and J01CA)
- Subjects requiring early intervention with antibiotic therapy due to the severity of diarrhea.

- Challenge Solicited Systemic Events: Selected solicited adverse events are assessed at the day of challenge, Post Challenge Day 1 to 7 and at discharge for the severity of: Fever, Nausea, Bloating, Vomiting, Generalized Myalgia, Arthralgia, Abdominal Pain, Abdominal cramping, Malaise, Headache, Loss of appetite, Dizziness. These events will be tabulated by symptom as well as by symptom and time point. Furthermore, symptoms that started ≥120h after challenge and symptoms that started >120h after challenge will be tabulated separately.
- Number of colony forming units (cfu) of the challenge strain per gram of stool on days 2 and 4 after challenge (V6 and V8). Therefore, from the microbiology results as provided by the central laboratory only the quantitative culture result will be analyzed and other included parameters will only be listed.
- Subjects requiring IV fluids.
- Subjects developing moderate to severe diarrhea by ABO blood type.

5.2.2 Derivations and Definitions

- All efficacy endpoints derived from the stool log will only use entries that are within 120 hours after challenge.
- All endpoints based on stool data will be reviewed by the CEC. In case the CEC disagrees with the calculated parameters, the values judged more appropriate will be used in the statistical analysis.
- All stool samples of grade 3-5 will be considered diarrheal.
- Time to onset of diarrhea from the time of receiving the ETEC challenge will be calculated by using only the data from the stool log and will be calculated in hours. Therefore, the time point of onset will be the time of the first diarrheal stool sample.
- Time to onset of moderate or severe diarrhea from the time of receiving the ETEC challenge (in hours) will be calculated by taking the first stool sample that is part of the first 24 hour period that fulfills the definition of moderate or severe diarrhea.
- Duration of diarrhea) will be defined as time of the longest phase of loose stool output. A phase ends at the last loose stool that is followed by grade 0-2 stool. For every subject, the mean total weight of grade 3-5 stools is defined as the mean of all volumes of stool samples of grade 3-5 that were reported for the subject.
- Number of grade 3-5 stools per subject is the sum of grade 3-5 stools reported in the stool log.
- Total volume of diarrheal stools will be defined as the sum of all volumes of diarrheal stools reported for a subject.
- Subjects with early intervention with antibiotic therapy due to the severity of diarrhea will be defined as subjects for whom "Indication for antibiotic treatment" was "Subject met criteria for early treatment" in the Antibiotic Administration Record.
- IV fluids will be identified from the eCRF by filtering concomitant medications for ATC codes B05BB

- Disease severity score will be calculated as described in Porter, et al; An Evidenced-Based Scale of Disease Severity following Human Challenge with Enteroxigenic Escherichia coli; Plos one, 2016 (see Table 1).
    - For the component "objective signs", the emesis log will be used to determine the number of vomiting episodes in a 24 hour period (entries within 120 hours after challenge). Fever will be identified from the solicited AE documentation after challenge (up to Post Challenge Day 5).
    - For the component "subjective symptoms", severity of the solicited symptoms Nausea, Abdominal cramping, Malaise, Headache and Dizziness reported after challenge will be considered that were reported up to Post Challenge Day 5.
    - For the component "diarrhea score", the number of entries and the reported volume of stools of grade 3-5 (within 120 hours after challenge) as documented in the stool log will be considered.

Table 1: Disease severity score components (Porter, et al)

Parameter	Outcome		Score
Objective signs	>1 episode of vomiting/24 hrs ***OR*** any fever		2
	1 episode of vomiting ***AND*** no fever		1
	No vomiting ***AND*** no fever		0
Subjective symptoms	Moderate-severe lightheadedness ***OR***		2
	Severe: nausea, malaise, headache or abd cramps		2
	Mild lightheadedness ***OR***		1
	mild-mod: nausea, malaise, headache or abd cramps		1
	No 'subjective symptoms'		0
Diarrhea score (max 24 hr loose stools)	>1000 ml	>12 episodes	4
	>600 to ≤1000 ml	>7 to 12 episodes	3
	>400 to ≤600 ml	>4 to ≤7 episodes	2
	>0 to ≤400 ml	1 to 4 episodes	1
	No loose stools	No loose stools	0

Footnote: diarrhea score assigned by the highest score determined by either maximum 24 hour output volume or frequency.

6. IMMUNOGENICITY ANALYSIS

6.1 Data Points

Serum IgG and IgA is measured at Visit 1 / Day 1, Visit 3 / Day 15 (7 days after second vaccination), Visit 11 / 7 days after challenge and Visit 13 / 28 days after challenge. Fecal IgA is determined at Visit 0 (or Visit 1 if not collected at Visit 0), Visit 3, Visit 11 and Visit 13.

Analyses will be provided for the safety population including Day 1 and Day 15 results and for the challenge population including all time points. Summary statistics for absolute values as well as for absolute changes from Day 1 will be provided for the following parameters:

- Serum IgA against LT
- Seroconversion from pre-challenge of IgA against LT
- Serum IgA against CS17
- Seroconversion from pre-challenge of IgA against CS17
- Serum IgG against LT
- Seroconversion from pre-challenge of IgG against LT
- Serum IgG against CS17
- Seroconversion from pre-challenge of IgG against CS17
- ALS IgA against LT
- ALS IgA against CS17
- Fecal total IgA
- Fecal IgA against LT
- Fecal IgA against CS17
- Serum vibriocidal titer

The following analyses will be provided for the challenge population (including all measurement time points):

- Serum IgG by time point
- Serum IgA by time point
- Absolute change in Serum IgG since Day 1 by time point
- Absolute change in Serum IgA since Day 1 by time point

Specifications on immunogenicity tables and listings are provided in Section 8.

6.2 Derivations and Definitions

- The Lower limit of detection is the lowest detected value obtained from the results of the assays. This value will be divided by 2 and the central laboratory will assign it to any titers that are below the lower limit of detection. No imputations will be performed in the statistical analysis and numbers will be analyzed as reported by the laboratory.

- Seroconversion of serum IgA and IgG titer against LT and CS17, respectively, is defined as two-fold increase in titer between pre- and post-challenge specimens **and** a post-challenge reciprocal titer > 100.
- Positive ALS IgA response will be defined as a four-fold rise in antibody titers between pre and post challenge samples.
- Fecal IgA against CS17 [mg/ml] will be calculated by using the following formula: Adjusted CS17 IgA titer / Total IgA [ug/ml] * 1000

7. SAFETY ANALYSIS

All safety analyses will be prepared for the safety population.

7.1 Exposure

Vaccination and challenge details will be listed.

7.2 Adverse Events

7.2.1 Data Points and Analyses

The following sources are used for AE analysis:

1. Solicited adverse events after vaccination: For selected solicited AEs there is a Baseline Assessment (prior to treatment administration), Post-Administration Assessment and assessments for the day of vaccination, and Post Vaccination Day 1 to 6 for the severity of: Diarrhea, Fever, Nausea, Bloating, Vomiting, Generalized Myalgia, Arthralgia, Abdominal Pain, Abdominal cramping, Malaise, Headache, Loss of appetite, Dizziness, Fatigue, Urticaria, Rash, Chills
2. General adverse event log: Unsolicited adverse events and serious solicited AE are documented in the general AE log. Not only the severity but also causality, outcome and seriousness are assessed for these events.

Adverse events from source 1 will be tabulated by symptom, vaccination period and severity.

The following analyses will be provided for events reported in source 2:

- Adverse event overview prior Challenge (number of subjects with any unsolicited AE, any unsolicited AE related to vaccination, any SAE, any unsolicited SAE, any solicited SAE etc.)
- Adverse event overview during entire study
- Adverse events by System Organ Class (SOC) and Preferred Term (PT) for specific types of AEs (e.g. unsolicited AE, unsolicited related AE, SAE) prior Challenge
- Unsolicited Adverse Events occurring within 30 days after 1st vaccination by SOC and PT
- AE by SOC and PT for specific types of AEs during the entire study
- Adverse events by severity, overall and for specific types of AEs prior Challenge
- Adverse events by severity, overall and for specific types of AEs during the entire study

One table will be prepared that shows all non-serious AE (solicited and unsolicited) from sources 1 and 2.

Specifications and details on TLFs are provided in Section 8.

7.2.2 Derivations and Definitions

- An AE will be considered to have occurred within 30 days after the 1st vaccination if the AE start date is within 30 days of the date of first vaccination (including AEs that started on the day of vaccination).

- To generate the table that combines the two AE sources, AEs from source 1 will be coded by using the codes as provided in Appendix 8.2. As only non-serious AE are to be included in the combined table, it has to be determined which solicited AE in source 1 was serious. This will be discussed in the DRM by considering source 1 together with the solicited SAE reported in source 2.
- For tables by severity subjects will only be counted once in highest grading category and events will be counted in each reported grading category.
- Tables for AE prior to challenge will use the period assignment in the CRF rather than the AE start date.

7.3 Laboratory Parameters

Laboratory data from scheduled visits will be tabulated. Listings will include results from unscheduled visits and scheduled visits.

7.3.1 Data Points

The following parameters are assessed in the study and will be included in the statistical analysis.

Hematology	Hemoglobin
Hematology	Hematocrit
Hematology	Platelets
Hematology	WBC
Hematology	Neutrophils
Hematology	Lymphocytes
Hematology	Basophils
Hematology	Monocytes
Hematology	Eosinophils
Chemistry	Sodium
Chemistry	Potassium
Chemistry	Chloride
Chemistry	Bicarbonate
Chemistry	Creatinine
Chemistry	Blood Urea Nitrogen (BUN)
Chemistry	Alanine Aminotransferase (ALT)
Chemistry	Glucose

For all parameters the following variables will be analyzed descriptively by time point:

- Absolute values
- Absolute change from Day 0
- Number of subjects with values above/below normal range

7.4 Other Safety Parameters

The following data will be analyzed:

- Vital Signs (Temperature, Blood Pressure, Pulse)

8. APPENDIX

8.1 Mock Tables for Interim Analysis

Table 1: Summary Table of Demographic Data (Challenge Population)				
	Statistic	VLA1701 (N=xxxx)	Placebo (N=xxxx)	Total (N=xxxx)
Sex				
male	n (%)	xx (xx.x)	xx (xx.x)	xx (xx.x)
female	n (%)	xx (xx.x)	xx (xx.x)	xx (xx.x)
Ethnicity				
Hispanic or Latino	n (%)	xx (xx.x)	xx (xx.x)	xx (xx.x)
Not Hispanic or Latino	n (%)	xx (xx.x)	xx (xx.x)	xx (xx.x)
Not reported	n (%)	xx (xx.x)	xx (xx.x)	xx (xx.x)
Unknown	n (%)	xx (xx.x)	xx (xx.x)	xx (xx.x)
Race				
American Indian or Alaska Native	n (%)	xx (xx.x)	xx (xx.x)	xx (xx.x)
Asian	n (%)	xx (xx.x)	xx (xx.x)	xx (xx.x)
Black or African American	n (%)	xx (xx.x)	xx (xx.x)	xx (xx.x)
Native Hawaiian or Other Pacific Islander	n (%)	xx (xx.x)	xx (xx.x)	xx (xx.x)
White	n (%)	xx (xx.x)	xx (xx.x)	xx (xx.x)
Other	n (%)	xx (xx.x)	xx (xx.x)	xx (xx.x)
Age at enrolment				
	Mean	xxx.x	xxx.x	xxx.x
	SD	xxx.xx	xxx.xx	xxx.xx
	Median	xxx.x	xxx.x	xxx.x
	Q1 / Q3	xxx.x / xxx.x	xxx.x / xxx.x	xxx.x / xxx.x
	Min / Max	xxxx / xxxx	xxxx / xxxx	xxxx / xxxx
	n	xx	xx	xx
More than one race may be reported for a subject				
n... number of subjects, percentages are based on N				

Table 2: Subjects with Moderate or Severe Diarrhea and Subjects with Diarrhea of any Intensity (Challenge Population)

	Statistic	VLA1701 (N=xxxx)	Placebo (N=xxxx)	Total (N=xxxx)	p- value
Primary endpoint: Subject experienced moderate or severe diarrhea within 120 hours after challenge					
	n (%)	xx (xx.x)	xx (xx.x)	xx (xx.x)	x.xxxx
	[95% CI]	[xx.x, xx.x]	[xx.x, xx.x]	[xx.x, xx.x]	
Subject experienced any diarrhea within 120 hours after challenge					
	n (%)	xx (xx.x)	xx (xx.x)	xx (xx.x)	x.xxxx
	[95% CI]	[xx.x, xx.x]	[xx.x, xx.x]	[xx.x, xx.x]	
Subjects with diarrhea within 120 hours after challenge by intensity					
Severe	n (%)	xx (xx.x)	xx (xx.x)	xx (xx.x)	
	[95% CI]	[xx.x, xx.x]	[xx.x, xx.x]	[xx.x, xx.x]	
Moderate	n (%)	xx (xx.x)	xx (xx.x)	xx (xx.x)	
	[95% CI]	[xx.x, xx.x]	[xx.x, xx.x]	[xx.x, xx.x]	
Mild	n (%)	xx (xx.x)	xx (xx.x)	xx (xx.x)	
	[95% CI]	[xx.x, xx.x]	[xx.x, xx.x]	[xx.x, xx.x]	

All cases assessed by independent Clinical Endpoint Committee

Subjects are counted only once, i.e. in highest grading category

p-value: Fisher's exact test, 95%-confidence intervals according to Altman

n... number of subjects, percentages are based on N

8.2 Coding Details for Solicited Adverse Events

Diarrhea	10012735	Diarrhoea	10017947	Gastrointestinal disorders
Fever	10037660	Pyrexia	10018065	General disorders and administration site conditions
Nausea	10028813	Nausea	10017947	Gastrointestinal disorders
Bloating	10000060	Abdominal distension	10017947	Gastrointestinal disorders
Vomiting	10047700	Vomiting	10017947	Gastrointestinal disorders
Generalized Myalgia	10028411	Myalgia	10028395	Musculoskeletal and connective tissue disorders
Arthralgia	10003239	Arthralgia	10028395	Musculoskeletal and connective tissue disorders
Abdominal pain	10000081	Abdominal pain	10017947	Gastrointestinal disorders
Abdominal cramping	10000081	Abdominal pain	10017947	Gastrointestinal disorders
Malaise	10025482	Malaise	10018065	General disorders and administration site conditions
Headache	10019211	Headache	10029205	Nervous system disorders
Loss of appetite	10061428	Decreased appetite	10018065	General disorders and administration site conditions
Dizziness	10013573	Dizziness	10029205	Nervous system disorders
Fatique	10016256	Fatigue	10018065	General disorders and administration site conditions
Urticaria	10046735	Urticaria	10040785	Skin and subcutaneous tissue disorders
Rash	10037844	Rash	10040785	Skin and subcutaneous tissue disorders
Chills	10008531	Chills	10018065	General disorders and administration site conditions