STATISTICAL ANALYSIS PLAN

AUTHOR(S):	Teesta Bose
PROJECT MANAGER:	Tamima Islam
SAP VERSION:	Final
DATE OF SAP VERSION	04 February 2019
SPONSOR:	Vaxart, Inc. 400 Oyster Point Blvd., Suite 222 South San Francisco, CA 94080
STUDY TITLE:	Evaluation of Infectivity and Illness of Norwalk GI.1 Virus Lot 001-09NV in the Human Challenge Model
PHASE OF STUDY:	Phase 1
PROTOCOL NUMBER:	VXA-G11-201.1
PROTOCOL VERSION/DATE:	Amendment 1, 21 September 2018

APPROVALS

Name and Title	Signature	Date
David Taylor, MD Chief Medical Officer Vaxart		
Kalyan Ghosh, Ph.D. Biostatistician WCCT Global		

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1. ABBREVIATIONS AND ACRONYMS

AE	Adverse Events
ALT	Alanine aminotransferase
ASC	Antibody secreting cell
BT_{50}	histo-blood group binding antigen blocking antibody titer
BUN	blood urea nitrogen
CRF	Case Report Form
ELISA	Enzyme-linked Immunosorbent Assay
GC	Genome copies
HBGA	Histo-blood group antigen
HBsAg	Hepatitis B surface antigen
HCV	Hepatitis C virus
HIV	Human Immunodeficiency Virus
ICH	International Conference on Harmonisation
IgA	Immunoglobulin A
IgG	Immunoglobulin G
NV	Norwalk Virus
NoV	Norovirus
NVG	Norovirus gastroenteritis
PBMC	Peripheral Blood Mononuclear Cells
PCR	Polymerase Chain Reaction
qRT-PCR	Quantitative Reverse Transcriptase Polymerase Chain Reaction
SAE	Serious Adverse Event/Serious Adverse Experience
VP1	Viral protein 1, major capsid or surface protein of viruses

2. INTRODUCTION

The purpose of this statistical analysis plan (SAP) is to outline in detail the statistical methods, data derivations, and presentations of data so that valid conclusions can be reached to address the study objectives outlined in the VXA-G11-201 protocol, amendment 1, dated 21 September 2018.

This SAP is being written with due consideration of the recommendations outlined in the most recent International Conference on Harmonization (ICH) E9 Guideline entitled Guidance for Industry: Statistical Principles for Clinical Trials and the most recent ICH E3 Guideline entitled Guidance for Industry: Structure and Content of Clinical Study Reports (CSR).

The planned analyses identified in this statistical analysis plan (SAP) may be included in regulatory submissions and/or future manuscripts. Exploratory analyses, not identified in this SAP, may be performed to support the clinical development program. The statistical analysis methods presented in this document will amend and/or supersede the statistical analysis methods described in the protocol. Any post-hoc or unplanned analyses that are performed but not identified in this SAP will be clearly identified in the CSR.

2.1. Responsibilities

WCCT will perform the statistical analyses for all clinical data collected. WCCT is responsible for production and quality control of all datasets, tables, figures, and listings.

2.2. Timing of Analyses

Only one final analysis after all subjects complete the study and subsequent database lock will be performed.

3. STUDY OBJECTIVES AND ENDPOINTS

3.1. Study Objectives

Primary Objective:

- Evaluate the infectivity and safety of the human challenge model with NV inoculum as measured by the number infected, number that become ill, and the number of SAEs.

  Infection status will be determined by: 1) virus detection in stool or emesis samples by quantitative reverse transcriptase-polymerase chain reaction (qRT-PCR) or 2) seroconversion through anti-NV antibody enzyme-linked immunosorbent assays (ELISAs).

Secondary Objectives:

- Determine the duration of viral shedding in stool and emesis samples after challenge.
- Solicited gastrointestinal symptoms
- Immunologic measurements of:

- anti-NV IgG and IgA

Exploratory Objectives:

- NV specific antibody response
- HGBA BT50.
- Antibody Secreting Cells (ASC) response

3.2. Endpoints

3.2.1. Primary Endpoints

- Occurrence of NVG within 7 days post-challenge (Study Day 9) in subjects for each dose group
- Occurrence of SAEs in subjects for each dose group

3.2.2. Secondary Endpoints

- Occurrence of any systemic solicited symptoms in subjects for each dose group (systemic solicited signs or symptoms are defined as diarrhea, vomiting, headache, nausea, fever, abdominal cramps or pain, abdominal gurgling or bloating, myalgia)
- Severity of NVG using the modified Vesikari Scale up to 7 days post-challenge
- Duration of NVG among challenged subjects up to 7 days post challenge
- Occurrence of evidence of NV infection (with challenge strain) in subjects up to 28 days post-challenge
- Percentage of subjects with seroconversion in serum anti-Norwalk pre-challenge to 28 days post-challenge
- Time to cessation of gastro-intestinal illness
- Duration of shedding.

3.2.3. Exploratory Endpoints

- Number (percent) of subjects with at least a 4-fold rise in NV-specific antibody titers post challenge.
- Number (percent) of subjects that seroconvert post-challenge, subdivided by illness status.
- Number (percent) of subjects with at least a 4-fold rise in HBGA BT50 from baseline compared to Day 28
- ASC IgA and IgG, Day 1 and Day 6 and/or Day 9
- Fecal IgA and saliva IgA, pre-challenge to post-challenge

4. STUDY DESIGN

A total of 16 eligible healthy young adults will be challenged with either the moderate or higher dose of NV strain Lot 001-09NV. Once informed consent has been obtained from the subject and s/he is determined eligible for the study, the subject will be admitted to the clinical research unit to receive the NV inoculum. Subjects will be monitored in isolation for at least 5 days (+ 1 day) after challenge.

During the inpatient stay, emesis (if available), stool, blood, and saliva samples will be collected from study subjects. Discharge from the research unit will be contingent upon: the absence of diarrhea and absence of moderate or high-grade objective reactogenicity (diarrhea, fever, and vomiting). After discharge subjects will have additional follow-up at days 9 ± 1, 14 ± 3, 28 ± 3, and 45 ± 3. The study subjects will be divided into 2 groups (Table 1). In Challenge 1, 8 subjects will receive the moderate dose and subsequently, if no safety flags are present, in Challenge 2, 8 subjects will receive the higher dose.

Table 1: Number of subjects to be challenged according to dose and study site

	Number of subjects		
Lot 001-09 NV	**Challenge 1**	**Challenge 2**	**Total**
Moderate dose, 3.6x105 GC	8		8
Higher dose, 1x106 GC		8	8
GC = genome copies			

4.1. Schedule of Evaluations

This study consists of three periods: an up to 60-day pre-screening to screening period, six day inpatient period (Day 1 to Day 6) and an outpatient/follow-up period (Day 9 to Day 45). The following assessments will be performed:

Table 2: Schedule of Study Visits and Evaluations

	Pre-Screening visit	Screening visit	Challenge phase (Inpatient)						Follow up Visits			
Study Day	-60 to -Screening	-30 to -3	1 Pre	2 Chal	3	4	5	6 /Disc	9	14	28	45
Compliance Range									± 1 d	± 3 d	± 3 d	± 3 d
Visit	00A	00B	00C	1	2	3	4	5	6	7	8	9
Outpatient	X	X							X	X	X	X
Inpatient stay			X	X	X	X	X	X				
Informed Consent	X	X										
ABO blood typing	X											
Saliva test for secretor status	X											
Demographics	X	X										
Weight, Height, BMI	X	X	X	X	X	X	X	X				
Eligibility criteria	X	X										
Medical interview		X	X	X	X	X	X	X	X	X	X	X
Physical Exam, (X) = targeted		X	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	
Vital signs		X	X	X	X	X	X	X	X	X	X	
Concomitant medications		X										
ECG		X										
Screen for HIV, HBsAg and HCV		X										
Safety laboratory evaluation		X	X					X				
Drug screen (urine, alcohol breath)		X	X									
Stool sample for enteric pathogens and occult blood		X										
Pregnancy test (females)		X	X								X	
Challenge				X								
Symptom grading				X	X	X	X	X				
Adverse events				X	X	X	X	X	X	X	X	X
Stool weighing/grading				X	X	X	X	X	X	X	X	
Emesis weighing				X	X	X	X	X				
Norovirus shedding (fecal/emesis)				X	X	X	X	X	X	X	X	
Discharge from inpatient								X				
Diary card (issue and/or return)								X	X			
Phone interview												X
Immunology												
Serum sample (antibody)			20 ml								20 ml	
Whole blood sample (PBMCs)			60 ml					60 ml	60 ml			
Fecal sample (IgA)			X								X	
Saliva sample (IgA)			X								X	
Assay Description												
qRT-PCR, Fecal	-	-	X	X	X	X	X	X	X	X	X	
qRT-PCR, Emesis	-	-		X	X	X	X	X				
VP1 IgG/IgA ELISA, Serum	-	-	X								X	

	Pre-Screening visit	Screening visit	Challenge phase (Inpatient)						Follow up Visits			
Study Day	-60 to -Screening	-30 to -3	1 Pre	2 Chal	3	4	5	6 /Disc	9	14	28	45
Compliance Range									± 1 d	± 3 d	± 3 d	± 3 d
Visit	00A	00B	00C	1	2	3	4	5	6	7	8	9
Assay Description (Exploratory)												
BT50, Serum	-	-	X								X	
ASC (IgG+IgA), PBMC	-	-	X					X	X			
VP1 IgA ELISA, Fecal	-	-	X								X	
VP1 IgA ELISA, Saliva	-	-	X								X	

a. A serum pregnancy test will be performed at screening and a urine pregnancy test will be performed before challenge and on Day 28 on all Females.
b. Screening laboratory to include: Complete Blood Count (CBC); Chemistry panel for ALT, Creatinine, Albumin, Total Bilirubin, Sodium, Potassium, Bicarbonate, Chloride, and BUN; Serology for HBsAg, anti-HCV, and HIV; Blood Typing; and Urinalysis
c. Safety laboratory to include: Complete blood count (CBC) with differential and Chemistry panel for ALT, Creatinine, Albumin, Total Bilirubin,
Sodium, Potassium, Bicarbonate, Chloride, and BUN
d. Height will only be collected at Screening Visit to calculate BMI. Measure body Weight only during Inpatient Challenge phase.
e. The observation and management of norovirus illness and stool grading and stool cultures are intended to be performed throughout the post- challenge inpatient period, until discharge criteria are satisfied
f. At Day 28 visit, if the stool specimen is positive for norovirus, subject will return to clinic weekly until they test negative or through Day 45.

ASC = antibody secreting cell; BUN = blood urea nitrogen; PBMC = peripheral blood mononuclear cell; ELISA = enzyme-linked immunosorbent assay; BT_{50} = histo-blood group binding antigen blocking antibody titers; qRT-PCR = quantitative reverse transcriptase-polymerase chain reaction; VP1 = viral protein 1

4.2. Interim Analyses

No interim analyses are planned for this study.

5. STUDY DOSE

The study doses are moderate or higher dose of NV strain Lot 001-09NV. In all data displays, treatment will be labeled as follows: “NV low” and “NV high”.

5.1. Method of Assigning Subjects to Dose Groups

This is an open label study. The two challenge cohorts will be enrolled sequentially.

5.2. Masking and Unmasking

This is an open-label study, so there is no masking in this study.

6. SAMPLE SIZE

A total of 16 eligible healthy adults will be recruited. We expect to screen more than 50 secretor-positive individuals to identify 16 that meet all the study inclusion and exclusion criteria. The study subjects will be divided into 2 groups. In Challenge 1, 8 subjects will receive the moderate dose and subsequently, if no safety flags are present, in Challenge 2, 8 subjects will receive the higher dose.

7. ANALYSIS POPULATIONS

The results from this study will be presented using the following populations:

For purposes of analysis, the following populations are defined:

Population	Description
All Subjects	All subjects who sign the Informed Consent Form (ICF)
Safety Population	The safety population includes all subjects who have received NV inoculum. Subjects in the Safety population will be analyzed as treated.

All statistical analyses will be performed for the Safety Population.

8. GENERAL ASPECTS OF THE STATISTICAL ANALYSIS

The following general rules will be followed for the analysis specified in this SAP.

Quantitative variables will be summarized using the following descriptive summary statistics: the number of subjects (n), mean, SD, median, minimum value (min), and maximum value (max).

Qualitative variables will be summarized using counts and percentages.

Baseline value will be defined as the last non-missing measurement (scheduled or unscheduled) prior to receiving NV inoculum. In most cases, this will be data from Study Day 1 Pre-dose (also referred as Visit 00C).

Unscheduled visits: Subject data obtained during unscheduled visits/assessments will not be summarized but will be included in subject data listings only; except for the analysis of maximum values and maximum changes from baseline. Unscheduled visit values will not be used to impute missing scheduled visit values, except for baseline calculation.

Outliers: Formal statistical analyses will not be performed to detect and/or remedy the presence of statistical outliers.

Study Day 2 is defined as the day the subject is exposed to NV inoculum. All study days are determined relative to the day of exposure to NV inoculum.

Duration on study for each subject is calculated as: the last day of observation - date of NV inoculum +1.

Treatment-emergent period is defined as the period at or after dosing of NV inoculum to the

end of the study.

Coding: For the purpose of summarization, concurrent therapies, and AEs will be coded to the latest versions of Medical Dictionary for Regulatory Activities (MedDRA) and World Health Organization Drug dictionaries (WHODrug), as appropriate.

9. DEMOGRAPHIC AND BASELINE CHARACTERISTICS

9.1. Subject Disposition

Subject disposition will be presented for all subjects enrolled.

Number and percentage of subjects in the following categories will be summarized as appropriate:

- Safety Population
- Study completion status - Did not complete all periods and Completed the study

Additionally, a summary on primary reason for discontinuations will be produced

A listing will be presented for Subject Disposition.

9.2. Demographic and Baseline Characteristics

The demographic and baseline characteristics will be summarized with respect to sex (M or F), age (years), race, ethnicity, height, weight, BMI, blood type (O or A), and, saliva secretor status by treatment group.

This summary will be calculated for Safety Population.

9.3. Prior and Concomitant Medication

Medications used in this study will be coded by using the World Health Organization Drug Dictionary Enhanced and categorized as follows:

- **Prior medication** is any medication that started before dosing of NV inoculum, regardless of when it ended.
- **Concomitant medication** is any medication received at or after the dose of NV inoculum, medication that was received before dosing of NV inoculum and continued, or medication with missing stop date.

A given medication can be classified as a prior medication or a concomitant medication or both. If a medication has a missing or partial missing start/end date or time and it cannot be determined whether it was taken before dosing of NV inoculum or concomitantly, it will be considered as both prior and concomitant.

Prior medication and concomitant medication will only be listed.

10. SAFETY

Safety analyses will be based on the Safety Population, and the safety summaries will be presented by dose groups. All safety assessments, including AEs, vital sign measurements, and clinical laboratory information will be listed.

10.1. Norovirus Gastroenteritis (NVG)

NVG is a composite endpoint for the analysis of clinical illness and is defined as meeting one or more definitions of Acute Gastroenteritis (AGE) **and** one or more of the definitions of NV Infection.

AGE:

- Diarrhea: ≥ 3 loose or liquid stools (i.e. grade 3 or higher stool) or > 400 grams of loose or liquid stools produced in any 24-hour period **OR**
- Vomiting: ≥ 2 vomiting episodes (i.e. grade 1 or higher vomiting) in any 24-hour period **OR**
- One vomiting episode plus any loose or liquid stool in any 24-hour period **OR**
- One vomiting episode plus at least 2 of the following 5 events (i.e. grade 1 or higher event):
    - headache,
    - nausea,
    - oral temperature ≥ 37.6°C (i.e. fever),
    - abdominal cramps or pains,
    - abdominal gurgling or bloating or myalgia

  in any 24-hour period.

NV Infection:

- NV infection as detected by qRT-PCR in one or more post-challenge stool or emesis samples through Day 7 post-challenge (Study Day 9).
- IgA/IgG ELISAs for anti-NV, ≥ 4-fold rise in titer in serum on Challenge Day 28 compared to pre-challenge

The number and percentage of subjects with NVG, among challenged subjects, during the inpatient phase, will be calculated by dose group

Time to onset of NVG and duration of NVG will be determined by dose group using diarrhea as the primary definition of NVG.

Calculation of the onset and duration of illness

- ONSET of illness will be defined as the time between NV challenge and the first diarrheal stool (defined as the passage of a ≥ grade 3 stool).
- DURATION of illness will be defined as the time between the first diarrheal stool and the last diarrheal stool (defined as the passage of a ≥ grade 3 stool).

Calculation of the onset and duration of infection

- ONSET of infection will be defined as the time between NV challenge and the first PCR positive stool.
- DURATION of infection will be defined as the time between the first PCR positive stool and the last PCR positive stool.

10.2. Systemic Solicited Signs or Symptoms

Systemic solicited signs or symptoms are defined as: diarrhea, vomiting, headache, nausea, fever, abdominal cramps or pain, abdominal gurgling or bloating, myalgia

The number and percentage of subjects for each specific systemic solicited signs or symptoms will be provided. In addition, a summary of maximum severity for each systemic solicited signs or symptoms will be provided by dose group and for NVG subjects.

10.3. Modified Vesikari Score:

Severity of NVG during the inpatient phase, using the modified Vesikari Scale will be summarized by dose group.

10.4. Other Secondary Endpoints:

Percentage of subjects with seroconversion in serum anti-Norwalk pre-challenge to 28 days post-challenge will be reported by dose groups. Based on PCR assay, duration of shedding will be calculated as,

duration of shedding = last date – first date +1,

and described using summary statistics by dose group.

10.5. Exploratory Endpoints

Number (percent) will be reported for

- subjects with at least a 4-fold rise in NV-specific antibody titers post challenge.
- subjects that seroconvert post-challenge, subdivided by illness status.
- subjects with at least a 4-fold rise in HBGA BT50 from baseline compared to Day 28

Descriptive statistics will be provided for

- HBGA BT50, Baseline and Day 28
- ASC IgA and IgG, Day 1 and Day 6 and/or Day 9
- Fecal IgA and saliva IgA, pre-challenge to post-challenge

10.6. Adverse Events

All AEs will be coded using the latest version of the MedDRA. Adverse events are only recorded in the eCRF after dosing.

The number and percent of subjects with at least one serious AE (SAE) will be presented by dose group. In addition, SAE at the subject and event level by System Organ Class (SOC) and Preferred Term (PT).

Similarly, the number and percent of subjects with at least one AE will be reported by dose group.

Additional summaries of AEs will be provided by relation to NV infection, by maximum severity and whether led to subject withdrawal. These summaries will also be presented by SOC and PT within each SOC by dose group. If a subject has multiple AEs coded to the same PT within the same SOC, the subject will be counted once under the maximal severity.

All AEs will be presented in a subject listing

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10.7. Pregnancy Test

A serum pregnancy test will be conducted for females of childbearing potential at screening (Visit 00B), at Day1 Pre-dose and at Day 28, and may be conducted at an Unscheduled Visit (e.g. early termination visit).

A listing of pregnancy test results will be generated.

10.8. Clinical Laboratory Assessments

Clinical laboratory parameters including complete blood count with differential and serum chemistry and urinalysis will be summarized by dose group and visit. Baseline values, the values at each subsequent visit, and changes from baseline will be summarized for each of the quantitative laboratory assessments by dose.

10.9. Vital Signs

Vital signs (including the assessments of systolic and diastolic blood pressure, heart rate and respiratory rate and temperature), and their change from baseline will be summarized for each scheduled visit and time point by dose group.

All data including unscheduled visits will be listed.

10.10. Physical Exam

Physical exam findings will be presented in a subject listing.

10.11. ECG

ECG findings collected at screening visit will be presented in a subject listing.

11. APPENDIX: ANALYSIS PRESENTATION CONVENTIONS

Post-text tables and listings will be prepared in accordance with the current ICH Guidelines. The information and explanatory notes to be provided in the "footer" or bottom of each table and listing will include the following information:

1. Date and time of output generation;
2. SAS® program name, including the path that generates the output;
3. Any other output specific details that require further elaboration.

In general, row entries in tables are made only if data exists for at least one subject (ie, a row with all zeros will not appear). The only exception to this rule applies to tables that list the termination status of subjects *(*eg, reasons for not completing the study). In this case, zeros will appear for study termination reasons that no subject satisfied.

The treatment and subject number will be included in all data listings. All listings will be sorted by study treatment, subject number, and visit date, as applicable. Subject listings will also include the number of days relative to the exposure to NV inoculum, if applicable.

This section details general conventions to be used for the statistical analyses. The following conventions will be applied to all data presentations and analyses.

- The number and percentage of responses will be presented in the form XX (XX.X%).
- All summary tables will include the analysis population sample size (ie, number of subjects).
- Date variables will be formatted as DDMMMYYYY for presentation.
- SAS® Version 9.3[1] or higher will be the statistical software package used for all data analyses.

12. REFERENCES

1. SAS Institute Inc., SAS® Version 9.3 software, Cary, NC.