Statistical Analysis Plan (SAP)

Protocol Title:	A Phase 1b, open-label, boost-optimization study of an adenoviral-vector based oral norovirus vaccine (VXA-G1.1-NN) expressing GI.1 VP1 administered orally to healthy adult volunteers
Protocol Number:	VXA-NVV-105
Protocol Version, Date:	Original v1.0, 31-MAR-2021
ICON GPHS ID:	4559-0016
Document Version, Date:	Final v2.0, 18-AUG-2021
Prepared by:	Chang Liu, PhD Senior Biostatistician
On behalf of:	Vaxart Biosciences, Inc. 170 Harbor Way, Suite 300 South San Francisco, CA 94080

Signature Page

Prepared at ICON GPHS by:	
Chang Liu, PhD Senior Biostatistician	
Signature	Date

Approved at ICON GPHS by:	
Toana Kawashima, MS Manager, Biostatistics	
Signature	Date

Approved at Vaxart Biosciences by:	
Shanita Crawford Senior Global Clinical Trials Manager	
Signature	Date

Consulting Biostatistician at Vaxart Biosciences	
Jeffery A. Davidson, PhD	
Signature	Date

Revision History

Version	Date	Revisions
0.1	15-APR-2021	Initial draft
0.2	21-APR-2021	Revised draft after incorporating internal comments.
0.3	30-APR-2021	Revised draft after incorporating sponsor's comments.
1.0	03-MAY-2021	Finalized SAP after sponsor review.
1.1	23-JUL-2021	Revised SAP to incorporate analysis (responder analysis and ANCOVA) for immunogenicity endpoints and some additional internal comments.
2.0	18-AUG-2021	Finalized SAP after reviewing and accepting sponsor's edits and comments.

Contents

List of Abbreviations

Abbreviation	Explanation
Ad5	Adenovirus type 5
AE	Adverse event
AESI	Adverse event of special interest
ALT	Alanine transaminase
ANCOVA	Analysis of covariance
ATC	Anatomical therapeutic chemical
ASC	Antibody secreting cells
AST	Aspartate aminotransferase
BMI	Body mass index
BT50	Blocking titer 50
BUN	Blood urea nitrogen
CPK	Creatine phosphokinase
CRF	Case report form
CSR	Clinical study report
DIC	Disseminated intravascular coagulation
ECG	Electrocardiogram
eCRF	Electronic case report form
ELISA	Enzyme-linked immunosorbent assay
ELISpot	Enzyme-linked immune absorbent spot
EOS	End of study
ER	Emergency room
ET	Early termination
FAS	Full analysis set
GCP	Good clinical practices
GM	Geometric mean
GMFR	Geometric mean fold rise
GMT	Geometric mean titer
GPHS	Government & Public Health Solutions
HBGA	Histo-blood group antigen
HBsAg	Hepatitis B surface antigen
HCV	Hepatitis C virus
HIV	Human immunodeficiency virus
ICH	International Council for Harmonization
IgA	Immunoglobulin A
IgG	Immunoglobulin G
IQR	Interquartile range
IU	International units
LS-GMR	Least square geometric mean ratio
LSM	Least square mean
MedDRA	Medical dictionary for regulatory activities
MSD	Meso scale discovery
NOCI	New onset of chronic illness
PBMC	Peripheral blood mononuclear cells
PI	Principal investigator
PP	Per protocol
PT	Prothrombin time
PTT	Partial thromboplastin time
SAE	Serious adverse event

Abbreviation	Explanation
SAP	Statistical analysis plan
SD	Standard deviation
SI	Standard international
SOC	System organ class
SoE	Schedule of Events
TLF	Tables, listings and figures
ULN	Upper limit of normal
VP1	Vaccine protein 1
WBC	White blood cell

1.0 Introduction

The purpose of this Statistical Analysis Plan (SAP) is to provide detailed descriptions of the statistical methods, data derivations, and data displays for the study protocol VXA-NVV-105 Original "A Phase 1b, open-label, boost-optimization study of an adenoviral-vector based oral norovirus vaccine (VXA-G1.1-NN) expressing GI.1 VP1 administered orally to healthy adult volunteers" dated 31-MAR-2021 for final analysis. The table of contents and templates for the table, listing and figures (TLFs) will be produced in a separate document.

Any deviations from this SAP will be described and justified in the Clinical Study Report (CSR).

The preparation of this SAP has been based on International Council for Harmonisation (ICH) E9 and Good Clinical Practice (GCP) guidelines.

All data analyses and generation of TLFs will be performed using SAS 9.4® (or higher).

2.0 Study Objectives

2.1 Primary objective

To evaluate the immunogenicity of VXA-G1.1-NN with repeat-dose administration at Day 1 and varying boost schedules (Week 4, 8 or 12 post initial dose) in healthy adults aged 18-55, inclusive.

2.2 Secondary objective

To assess the safety and tolerability of VXA-G1.1-NN with repeat-dose administration at varying boost schedules (Week 4, 8 or 12) in healthy adults aged 18-55, inclusive.

2.3 Exploratory objectives

- To assess additional safety parameters
- To assess long-term safety of VXA-G1.1-NN through 6 months after last vaccination
- To assess additional immunogenicity parameters of VXA-G1.1-NN

2.4 Safety objectives

Safety objectives are included as part of the secondary and exploratory objectives.

3.0 Study Design

3.1 General study design

This is a Phase 1b, open-label, boost-optimization study to evaluate the immunogenicity, safety and tolerability of VXA-G1.1-NN with repeat-dose administration at Day 1 and varying boost schedules in healthy

adult subjects aged 18 – 55. Thirty subjects will be enrolled to one of the three treatment cohorts (10 subjects in each cohort) with varying boosting vaccination schedules at Week 4, 8 or 12, respectively, post the initial vaccination on Day 1 such that each subject will receive two doses of 1×10^{10} IU ± 0.5 log in total. See study protocol Section 5 for inclusion and exclusion criteria.

On Day 1, participants will be enrolled sequentially in order of eligibility. Participants may be assigned to cohorts based on their availability for the second dose (boost). Cohorts 3 and 2 will be enrolled prior to Cohort 1 to optimize the overall study timeline.

Table 1: Treatment Arm, Doses and Sample Sizes

Group	Study Drug	Dose (IU ±0.5 log)	Doses	Dosing Schedule	Subjects
Cohort 1	VXA-G1.1-NN	$1x10^{10}$	2	Day 1 & Week 4	10
Cohort 2	VXA-G1.1-NN	$1x10^{10}$	2	Day 1 & Week 8	10
Cohort 3	VXA-G1.1-NN	$1x10^{10}$	2	Day 1 & Week 12	10
				Total	**30**

The study will include a Screening period, Study Period 1, Study Period 2, and a Follow-up Period:

- Screening Period (Day -45 to -1)
- Study Period 1 (Day 1 to 4 weeks post initial vaccination)
- Study Period 2 (4 weeks from the second vaccination: Week 4 to Week 8 for Cohort 1; Week 8 to Week 12 for Cohort 2; Week 12 to Week 16 for Cohort 3)
- Follow-up Period (6 months post the second vaccination for each cohort: Month 7 for Cohort 1, Month 8 for Cohort 2 and Month 9 for Cohort 3)

3.2 Randomization and blinding

As VXA-NVV-105 is a Phase 1b, open-label, boost-optimization study to determine the immunogenicity, safety and tolerability of vaccine VXA-G1.1-NN at dose level 1×10^{10} IU ± 0.5 log with varying boosting schedules, neither randomization nor blinding will be applicable for this study. Both subjects and investigators will be aware of individual treatment assignment.

3.3 Study treatments and assessments

Subject participation will last for about 7 months for Cohort 1 (4-week boost vaccination), 8 and 9 months for Cohorts 2 and 3 respectively (Cohort 2: 8-week boost vaccination; Cohort 3: 12-week boost vaccination). Subjects will be followed for 4 weeks after each dose, and through 6 months following the boosting dose. Following confirmation of eligibility during the screening period, subjects will be enrolled and receive their first

dose of study vaccine on Day 1 and their second dose of study vaccine on Day 29, Day 57 or Day 85 depending on their assigned cohort if deemed eligible after pre-dose safety assessments.

Subjects will record any potential symptoms of reactogenicity daily for 1 week after each dose and return to the site to have safety assessments and samples collected for evaluation of immunogenicity. Subjects deemed ineligible for the second vaccination due to acute illness or new medical condition may be re-assessed and have a delayed second vaccination within 1 week of their scheduled vaccination visit if the condition resolves. If a subject remains ineligible after the re-assessment, they will complete an Early Termination (ET) visit and then enter the scheduled follow-up period per cohort assignment as outlined in the schedule of events (SoE). All subjects will enter the follow-up period 4 weeks after their last vaccination and will be monitored for serious adverse events (SAEs), adverse events of special interest (AESIs) and new onset of chronic illness (NOCI) through the End of Study. Subjects will also have samples collected for evaluation of immunogenicity as scheduled in SoE.

A detailed description of procedures and assessments to be conducted during the active period of this study is summarized in the Schedule of Events in Table 2 below, with those during the follow-up period summarized in Table 3.

Table 2 – Schedule of Events (Active Period)

Active Period										
	Screen	Study Period 1 – Cohorts 1, 2, 3								
				Study Period 2 – Cohort 1						
						Study Period 2 – Cohort 2				
								Study Period 2 – Cohort 3		
Study Day	**-45d to –1 d**	**Day 1 (Wk 0)**	**Day 8 (Wk 1)**	**Day 29 (Wk 4)**	**Day 36 (Wk 5)**	**Day 57 (Wk 8) /ET[1]**	**Day 64 (Wk 9)**	**Day 85 (Wk 12)/ET[2]**	**Day 92 (Wk 13)**	**Day 113 (Wk16)/ET[3]**
Visit Window (days)		n/a	0	**±2**	0	**±2**	0	**±2**	0	**±2**
Informed consent	X									
Inclusion/Exclusion	X	X		X						
Demographics	X									
Medical history	X									
Stool for occult blood	X									
Blood Sample (HBsAg, HCV, HIV)	X									
Urine drug screen	X									
Serum Pregnancy Test[a]	X	X		X[1]		X[2]		X[3]		
Physical examination	X	X[b]	X[b]	X[b]	X[b]	X[b]	X[b]	X[b]	X[b]	X[b]
Vital Signs	X	X	X	X	X	X	X	X	X	X
Safety laboratory tests	X	X[c]	X	X	X	X	X	X	X	X
Urinalysis	X	X	X	X	X	X	X	X	X	X
COVID vaccination status	X									
Vaccination		X		X[1, d,e]		X[,2, d,e]		X[3, d,e]		
Dispense Solicited Symptom Diary		X[1,2,3]		X[1]		X[2]		X[3]		
Review & Collect Solicited Symptom Diary			X		X[1]		X[2]		X[3]	
Review prior & concomitant medication	X	X	X	X	X	X	X	X	X	X
Query for AEs, SAEs, AESIs andNOCIs		X	X	X	X[1,f]	X[1,f]	X[2,f]	X[2,f]	X[3,f]	X[3,f]
Sample Collection Immunogenicity Assessments										
Serum		X[1,2,3[g]]		X[1,2,3]		X[1,2]		X[2,3]		X[3]
Whole blood (Flow-Based)		X[1,2,3]	X[1,2,3]	X[1]	X[1]	X[2]	X[2]	X[3]	X[3]	
Whole blood (PBMC)		X[1,2,3]	X[1,2,3]	X[1]	X[1]	X[2]	X[2]	X[3]	X[3]	
Nasal Swab (Sam Device)		X[1,2,3]		X[1,2,3]		X[1,2]		X[2,3]		X[3]
Saliva Sample		X[1,2,3]		X[1,2,3]		X[1,2]		X[2,3]		X[3]

[1] = Cohort 1; [2] = Cohort 2; [3] = Cohort 3.

[a] For women of childbearing potential. Test at Day 1 and at second dose (boost). Test at 2nd Study drug administration visit can be serum or urine test.

[b] Targeted.

[c] If screening lab tests are performed within 2 days prior to dosing, no need to repeat at Day 1.

[d] Assess eligibility to receive second dose. Participants with acute illness or new medical condition may be re-assessed, and if the condition resolves, have a delayed 2nd dose within 1 week of scheduled visit.

[e] If participants are not eligible to receive their 2nd dose, they immediately enter the follow-up period.

[f] AEs will only be collected for 4 weeks following each study drug administration.

[g] An aliquot of serum collected at baseline will be stored for testing (e.g. Platelet Factor 4 [PF4] antibody ELISA) should an AESI related to blood clots be reported anytime during the study period.

Table 3 – Schedule of Events (Follow-up Period)

Follow-Up Period						
	COHORT 1		COHORT 2		COHORT 3	
Study Day	**Months 3, 4, 5, 6**	**Month 7 (Wk 28)/ EOS**	**Months 4, 5, 6, 7**	**Month 8 (Wk 32)/EOS**	**Months 5, 6, 7, 8**	**Month 9 (Wk 36)/EOS**
Telephone Visit	X		X		X	
Visit Window (days)	**±7**	±7	±7	±7	±7	±7
Query for SAEs, AESIs and NOCIs	X	X	X	X	X	X
Sample Collection Immunogenicity Assessments						
Serum		X		X		X
Whole blood (PBMC)[a]		X		X		X
Nasal Swab (Sam Device)		X		X		X
Saliva		X		X		X

AE, adverse event; AESI, Adverse event of special interest; EOS, End of Study; ET, Early Termination; HCV, Hepatitis C Virus; HIV, Human Immunodeficiency Virus; NP, Nasopharyngeal; NOCI, New Onset of Chronic Illness; PBMC, peripheral blood mononuclear cells; SAE, serious adverse event.

[a] Optional.

4.0 Study Endpoints

4.1 Primary endpoints

- Number of VP1 specific IgA antibody secreting cells (ASC) by enzyme-linked immunospot (ELISpot) by treatment group and visit
- Geometric mean titer (GMT) and geometric mean fold rise (GMFR) over the initial GMT of Norovirus G1.1 histo-blood group antigen (HBGA) blocking antibodies (BT50) by treatment group and visit
- Geometric mean (GM) and GMFR over the initial results of VP1 specific serum IgG by Meso Scale Discovery (MSD) by treatment group and visit

4.2 Secondary endpoints

- Frequencies, durations, and severities of solicited symptoms of reactogenicity (local, systemic) for 1 week following each dose of study drug by treatment group
- Frequencies, durations, and severities of unsolicited AEs, SAEs, AESIs and NOCIs through each active study period (4 weeks post each vaccination) by treatment group
- Number and percentages of subjects with ≥2-, 3-, 4-fold increase over baseline titer of Norovirus G1.1 HBGA BT50 results by treatment group and visit
- Geometric Least Square Mean (LSM) of post-dose Norovirus G1.1 HBGA BT50 titer by treatment group and visit adjusted for baseline titer level
- Least Square Geometric Mean Ratio (LS-GMR) of post-dose titer Norovirus G1.1 HBGA BT50 between treatment groups by visit adjusted for baseline titer level

4.3 Exploratory endpoints

- Frequencies of clinically significant abnormalities in laboratory parameters (chemistry, hematology and urinalysis) at 1 week after each study drug dose and in vital signs immediately following each dose by treatment group
- Frequencies, durations, and severities of all SAEs, AESIs and NOCIs through 6 months after the last vaccination by treatment group
- The following additional immunogenicity parameters will be analyzed by treatment group and visit, and will be reported in the CSR:
    - Number of VP1 specific IgG ASC
    - GM and GMFR over the initial results of VP1 specific serum IgA

Additional exploratory immunogenicity assays not listed above may also be performed to further evaluate the activity of the VXA-G1.1-NN vaccine candidate. The data from such exploratory assays will be reported outside the scope of this SAP.

5.0 Sample Size and Power

No formal sample size calculation was performed for the study. Thirty subjects in total (10 in each cohort) are planned to be enrolled based on experience of a typical Phase I vaccine study.

6.0 Analysis Populations

6.1 Full Analysis Set (FAS)

The FAS will consist of all subjects who have received at least one dose of vaccine VXA-G1.1-NN. Subjects will be included in the analysis according to the cohort to which they are assigned. Subjects who have been enrolled but never received the study vaccine will be excluded from the analysis.

6.2 Safety population (Safety)

Safety population will consist of all subjects who receive at least one dose of study vaccine and will be analyzed according to the cohort to which they belong according to the actual dosing schedule. Subjects who have been enrolled but never received the study vaccine will be excluded from the analysis.

6.3 Per-Protocol population (PP)

The per-protocol population will consist of all subjects in the FAS who received both vaccine doses and are free from major protocol violations that warrant exclusion. Subjects will be included in the analysis according to the cohort to which they are assigned.

6.4 Protocol deviations/violations and exclusions from analysis sets

All violations and exclusions of subjects from analysis sets will be identified and documented prior to data base lock and final analysis, through clinical review input provided by the sponsor, using the following sources of information:

- Supportive subject listings provided by the ICON GPHS statistician based upon data recorded in the CRF
- Protocol deviation logs provided by the site
- Protocol deviation listings provided by the Sponsor

Deviations from the protocol will be classified as to whether or not they are Major Deviations.

The major protocol deviations to be identified and to be included in the body of the CSR will include the following at a minimum, as well as others classified as key during review of all deviations observed:

- Failure to obtain informed consent
- Incorrect dose of vaccine
- Failure to meet eligibility criteria at baseline

7.0 Statistical Considerations and Analysis

7.1 Derived Variables

The below table provides the list of derived variables for demographic and baseline characteristics, various duration derivations, baseline derivation and other important derivations applicable for this study.

Table 4: Derived Variables

Variables	Formula
Demographic and Baseline Characteristics	
Age at informed consent (in years)	Integer ((date of informed consent – date of birth + 1) / 365.25)
Body Mass Index	Weight (kg) / height $(m)^2$
Derivation of Durations	
Study day at any visit	Date of interest – date of first dose of vaccine. One day is added if the difference is ≥ 0.
Duration of any events	End date of event – start date of event + 1
Baseline Derivations	
Laboratory baseline	The baseline value is defined as the last observation prior to or on the date of the first dose of study drug.
Change from baseline	Post baseline value – baseline
Percent change from baseline	[(Post baseline value – baseline) / baseline] * 100
Change from previous visit	Current value – previous value
Percent change from previous visit	[(Current value – previous value) / previous value] * 100
Other Derivations	
Geometric mean/geometric, mean titer/geometric mean concentration	Individual values will be transformed via the natural log, mean values along with 95% CIs will be calculated, which will be transformed back to the original scale via exponentiation.
Geometric mean fold rise	The mean values and 95% CIs of the log ratio of post dose assay values relative to baseline will be calculated, then transformed back to the original scale via exponentiation.
Geometric least square mean	Least square means and 95% CIs for each treatment group will be calculated from ANCOVA with immunogenicity assay result on log scale at post dose visit as a dependent variable, treatment group as a factor and baseline immunogenicity assay result on log scale as a covariate, which will be then transformed to the original scale via exponentiation.
Least square geometric mean ratio	Least square mean differences and 95% CIs among treatment groups will be estimated from the abovementioned ANCOVA model, which will be then transformed to the original scale via exponentiation.

7.2 Handling of missing data and outliers

The extent and pattern of missing data for primary, secondary, and exploratory endpoints will be summarized by cohort and visit date with frequencies and percentages. No imputations will be conducted for missing data other than dates. No outliers will be eliminated.

7.3 Missing data analysis methods

Imputation rules for missing or partial adverse event start/stop dates

- If the AE start date day is missing (month and year provided) then set the date to the first of the month, unless the month and year are the same as the first dose of study drug. In this case, set the date to the date of first dose.
- If the AE start date month is missing (year is provided) then set the month and day to January 1, unless the year is the same as the year of the first dose. In this case, set the date to the date of first dose.
- If the AE end date day is missing (month and year provided) then set the date to the last day of the month.
- If the AE end date month is missing (year is provided) then set the date to December 31 or current date, whichever is earlier.
- If the year of the AE start date or AE end date are missing, then a query to the site must be made to gather additional information. If the end date and start date are both missing, then no imputation will be done. If the start date remains missing but the end date is before first dose date, then the AE will be considered before treatment and it will not be recorded as an AE in CRF but included as medical history. If the end date is after the first dose, then the AE will be considered to have been treatment emergent.

Imputation rules for missing or partial medication start/stop dates

Start Date:

- If only day is missing, use the first day of the month.
- If day and month are missing, use the first day of the year.
- If day, month, and year are missing use the first day of the year with the same year as the first dose.

End Date:

- If only day is missing, use the last day of the month.
- If day and month are missing, use the last day of the year.
- If day, month, and year are missing assign ‘continuing’ status to the stop date.

8.0 Statistical Methods

8.1 General statistical conventions

All statistical procedures will be completed using SAS version 9.4 (or higher).

Continuous variables will be summarized using descriptive statistics, including number of subjects (n), mean, median, standard deviation (SD), interquartile ranges (IQR), minimum, and maximum.

For categorical variables, summaries will include counts of subjects and percentages. Percentages will be rounded to one decimal place.

For summary purposes, baseline will be defined as the last available pre-dose value. All summaries will be presented by treatment group, unless otherwise specified.

Immunogenicity titer data will be summarized using GMT/GMC and GMFR over baseline GMT/GMC. Immunogenicity data below the lower limit of quantification (LLOQ) will be summarized as LLOQ/2 in all relevant tables and will be displayed in listings in the format of source data. Immunogenicity data above the upper limit of quantification (ULOQ) will be summarized using ULOQ in all relevant tables and will be displayed in listings in the format of source data.

All subject data, including those derived, will be presented in individual subject data listings. Unless otherwise stated, unscheduled visit results will be included in date/time chronological order, within patient listings only. All listings will be sorted by subject ID, treatment group, study visit. Subject's sex and age will be also stated on each listing. Unless otherwise stated, data listings will be based on FAS.

Unscheduled data points will not be used in any summary tables separated by visit.

8.2 Subject disposition

Subject disposition information will be summarized by treatment group and overall. The number and percentage of subjects who are enrolled, who are eligible/ineligible to receive the second dose of vaccine, who complete the active period of the study, and who withdraw early from the study will be presented.

The primary reasons for ineligibility and early withdrawal will also be tabulated.

The number of subjects enrolled will be used as the denominator for the percentage calculation. Subject disposition will be listed.

The number and percentage of subjects in each analysis set will also be tabulated. A listing of each subject excluded from an analysis population will be listed as well as the reason why they were excluded from the population.

8.3 Protocol deviations

The number of subjects excluded from FAS, Safety, and Per-protocol analysis sets and reasons for exclusion will be summarized by treatment group and overall.

Population membership details will be listed, including reason for exclusion from each population.

Protocol deviation data are not captured in the clinical database and therefore will not be reported on individually within the scope of the SAP. Protocol deviations will be summarized in the final CSR.

8.4 Demographics and baseline characteristics

8.4.1 Demographics

Summary of demographics will be based on the FAS. Age at consent in years, height in cm, weight in kg and BMI (kg/m^2) will be summarized descriptively using mean, SD, median, IQR, and range. Sex at birth, race and ethnicity will be summarized using frequencies and percentages.

8.4.2 Baseline and disease characteristics

The baseline continuous characteristics (vital signs) will be summarized descriptively for the safety population using mean, SD, median, IQR, and range. The baseline categorical characteristics (results of physical exam, chemistry, hematology and urinalysis) will be summarized (normal vs abnormal) using frequencies and percentages for the safety population.

8.4.3 Medical history

A summary of medical history will be presented by system organ class (SOC) and preferred term using the Medical Dictionary for Regulatory Activities (MedDRA) version 24.0 by cohort at baseline.

8.4.4 Prior and concomitant medications

Medications used in this study will be coded by using the World Health Organization Drug Dictionary Enhanced (WHODrug version March 1, 2021).

Concomitant medication is defined as any prescription or over-the-counter preparation. Use of concomitant medication from 4 weeks before Day 1 through 4 weeks after the last dose administration must be recorded in eCRF from the subject's medical file along with reason for use, dates of administration including start and end dates, dosage information including dose and frequency. Medications for pre-existing medical conditions or required for a medical condition during the study are allowed if not considered exclusionary. Medications specifically prohibited in the exclusion criteria (Section 5.2 in study protocol) are not allowed during the active study period, unless deemed medically necessary by the investigator. The Sponsor's medical monitor (or designee) should be contacted if there are any questions regarding concomitant or prior medications.

Concomitant medications will be summarized descriptively using frequency tables by Anatomical Therapeutic Chemical (ATC) class (hierarchically by Level I, II and III) and cohort in the descending order of overall frequency. All concomitant medications will be presented in listings as well.

Details for imputing missing or partial start and/or stop dates of medication are described in Section 7.3.

8.5 Extent of exposure

Vaccines will be administered on-site for all subjects. All subjects will receive the first dose on Day 1. Subjects will receive the second dose on Day 29 for Cohort 1 (4-week boost vaccination), Day 57 for Cohort 2 (8-week boost vaccination), and Day 85 for Cohort 3 (12-week boost vaccination), respectively, allowing for a potential delay up to 1 week. Frequencies and percentages will be calculated for the number of doses received (1 dose or 2 doses), the presence of delay in administering the second dose, whether vaccination is administered per protocol along with reasons why vaccine administration is not per protocol.

8.6 Immunogenicity analysis

This section addresses the analysis methods for immunogenicity outcomes.

8.6.1 Analysis methods

Immunogenicity will be evaluated using cellular and humoral immune function assays from blood and mucosal (saliva and nasal swab) samples. Samples will be collected from all subjects according to the time points specified in the SoE.

Results of the following immunogenicity assay results will be summarized descriptively by treatment group and visit and will be reported in the final CSR. Specific endpoints are listed in Sections 4.1 and 4.3.

- VP1 specific IgA ASC by ELISpot
- Norovirus G1.1 HBGA BT50
- VP1 specific serum IgG by ELISA or MSD
- VP1 specific IgG ASC by ELISpot
- VP1 specific serum IgA by ELISA or MSD

Additional exploratory endpoints will be evaluated and reported outside the scope of this SAP.

Kolmogorov-Smirnov test will be used to examine the normality assumptions of the immunogenicity assay results in log scale for each treatment group. If normality assumptions are met for all treatment groups, pairwise comparisons will be performed between treatment groups using Student t-test; Wilcoxon rank sum test will be used if otherwise.

8.6.1.1 Responder Analysis

A responder analysis at each appropriate post-dose follow-up visit (see Table 1 and Table 2 for specific timings) will be conducted for the Norovirus G1.1 HBGA BT50. During active period, the numbers and percentages (with 95% CIs) of subjects with a ≥2-, 3- or 4-fold increase from baseline antibody levels will be summarized, respectively. Durability of immune responses will be evaluated using the safety follow-up period samples collected at 6 months post the second dose (Month 7 for Cohort 1, Month 8 for Cohort 2, and Month 9 for Cohort 3). Numbers and percentages of subjects with a ≥2-, 3- or 4-fold increase from baseline antibody levels at both 28 day post the second dose (Day 57 for Cohort 1, Day 85 for Cohort 2, and Day 113 for Cohort 3) and 6 month post the second dose will be summarized, where percentages will be calculated as of the number of subjects with a ≥2-, 3- or 4-fold increase from baseline antibody levels at 28 day post the second dose. Fisher's exact test will be used to compare the percentages of responders for each category among cohorts.

8.6.1.2 Analysis of Covariance (ANCOVA)

To account for the potential differences in baseline antibody level, ANCOVA will be used to analyze and compare the immunogenicity endpoints at each post-dose visits conditioning on baseline values for the Norovirus G1.1 HBGA BT50 assay. Separate models will be estimated at each post-dose visit, using log titer as a dependent variable, treatment group as a factor and baseline log titer as a covariate. LSMs for each cohort and differences in LSM between cohorts will be estimated from each model along with 95% CIs. Both LSMs for each cohort and the differences in LSM between cohorts along with their 95% CIs will be exponentiated to the original scale, resulting geometric LSMs for each cohort and LS-GMR between cohorts. In order to compare immunogenicity assay results among treatment groups, significance on 28 days post the second dose will be used. If the ANCOVA demonstrates overall significance on 28 days post the second dose, pairwise comparisons will be performed.

8.6.2 Multiplicity

There will be multiple outcomes pertaining to immunogenicity; however, each outcome will be analyzed separately, and no corrections will be made to account for multiplicity. Pairwise comparisons among cohorts will also be conducted; Holm-Bonferroni method will be used to control family-wise error rate, both adjusted and unadjusted p-values will be reported.

8.6.3 Treatment by center interaction analysis (multi-center study)

Not applicable since this is a single-center study.

8.7 Safety analysis

This section describes the safety analyses that will be conducted during the active and follow-up periods (i.e., the safety analyses on all data collected during the active and follow-up periods and all data collected in subjects who dropped-out during the active and follow-up periods).

All definitions relative to safety endpoints are detailed in Section 4.4.

Safety analyses will be conducted on the safety population and will be performed for all safety variables specified below.

All safety data will be summarized by cohort and study visit as well as by overall study population.

The safety analyses of changes from baseline to a specific time point in safety variables (e.g., vital signs) will only include subjects from the safety population who have data available at both the baseline and the time point under considerations unless otherwise specified.

8.7.1 Adverse events

All adverse events (AEs) (except for solicited AEs) will be classified by primary SOC and preferred term according to the Medical Dictionary for Regulatory Activities (MedDRA) Version 24.0

AEs will be categorized by solicited AEs, unsolicited AEs (including both AESIs and NOCIs) and SAEs, where the frequencies and durations of each will be summarized by cohort, severity and relatedness to study treatment.

Solicited AEs are predefined systemic signs and symptoms of reactogenicity for which the subjects are specifically questioned; including fever (any temperature 100°F or higher), headache, myalgia (muscle pain), abdominal pain, anorexia (defined as not eating), nausea, vomiting, diarrhea and malaise/fatigue. Frequencies and percentages for each solicited symptom will be calculated. Severities of solicited AEs will be graded according Appendix 1. Definitions of AE, AESI and NOCI are provided in the study protocol (refer to Tables 7 – 11 in the study protocol Section 10.3).

Unsolicited AEs will be summarized by SOC and preferred term for AESIs and NOCIs combined where the events will be sorted by descending overall frequency within each SOC and preferred term according to the total number of events.

Frequencies and percentages of SAEs will be summarized by treatment group, along with specifics including whether the AE is associated with a congenital anomaly or birth defect, whether the AE results in persistent or significant disability or incapacity, initial or prolonged hospitalization, and death, whether the AE is life-threatening, or medically important but not covered by other serious criteria.

The severity of AEs will be assessed by the clinician using a protocol-defined grading system. For events that are not listed in the protocol-defined grading system, refer to Table 12 in the study protocol Section 10.3 for classifying severity.

Relationships of AEs to study vaccine are categorized as not related, possibly related, probably related, and definitely related (refer to Table 17 in the study protocol Section 10.3.1 for details).

Details for imputing missing or partial start dates of adverse events are described in Section 7.2 of this SAP.

Where a subject has the same adverse event, based on preferred terminology, reported multiple times in the treatment period, the subject will only be counted once at the preferred terminology level in adverse event frequency tables.

Where a subject has multiple AEs within the same SOC in the treatment period, the subject will only be counted once at the SOC level in AE summary tables.

Listings will be provided for solicited AEs, unsolicited AEs (including both AESIs and NOCIs) and SAEs, where patient information, SOC and preferred term, durations, severities, relatedness to study treatment, outcomes, and actions will be included.

8.7.2 Clinical laboratory evaluations

Clinical laboratory evaluations include serum chemistry, hematology and urinalysis. For the purposes of summarization in both the tables and listings, all laboratory values will be presented in SI units. If a lab value is reported using a nonnumeric qualifier e.g., less than (<) a certain value, or greater than (>) a certain value, the given numeric value will be used in the summary statistics, ignoring the nonnumeric qualifier. Severity of laboratory results will be categorized as mild, moderate, severe and potentially life threatening according to Appendices 2 - 4 in current document.

All laboratory data obtained between screening visit and the end of study will be used for the laboratory safety analysis. Frequencies and percentages of subjects with abnormal laboratory results will be summarized for each laboratory test and further by individual laboratory parameters at each time point. Shift tables for hematology and chemistry laboratory assessments between baseline and each subsequent visit will be provided if applicable.

Abnormal laboratory results will be listed by treatment group and visit, including patient information, lab parameter, lab results along with normal ranges, and abnormality criteria.

8.7.3 Vital signs

Visit values and changes from baseline for vital sign measurements (blood pressure, oral temperature, heart rate and respiratory rate) will be summarized by cohort at each visit using descriptive statistics. Visit values will be calculated as the mean of all available measurements per parameter if multiple measurements are taken during the visit.

8.7.4 Physical examinations

Physical examinations will be conducted at baseline and all subsequent visits. At baseline, a complete physical examination will be conducted and results by body system and specific abnormalities along with clinical significance will be summarized by treatment group using frequencies and percentages. At subsequent visits, targeted and symptom-directed physical examinations will be conducted. Any changes from previous physical examination will be documented; frequencies and percentages of abnormal findings

will be summarized by treatment group; if deemed as adverse events, results will be listed accordingly by subject and summarized by treatment group at each visit.

8.7.5 Other safety assessments

Urine drug screen, tests for hepatitis B surface antigen, hepatitis C virus antibody, human immunodeficiency virus antibody types 1 and 2, stool for occult blood and alcohol screen will be administered at screening for all subjects. Women with childbearing potential will receive serum pregnancy test at screen, serum or urine pregnancy test on dosing visits. Results of the abovementioned safety assessments will be summarized via listings.

8.8 Follow-up Period Analysis

Following completion of the study Active Period for all enrolled subjects, the study database will be cleaned and locked then the CSR will be finalized. Safety and immunogenicity data collected during the safety follow-up period will be appended to the CSR via an addendum after completion of the study (6 months post the last vaccination), where tables and listings pertaining to SAEs, immunogenicity endpoints will be updated accordingly incorporating data collected during the safety follow-up period.

9.0 Changes to Planned Analysis from Study Protocol

None.

10.0 References

1. ICH E9 Statistical Principles for Clinical Trials (R1)

11.0 Appendices

Appendix 1 - Grading of Solicited Symptoms of Reactogenicity

Symptom	Grading				
	Normal 0	**Mild Grade 1**	**Moderate Grade 2**	**Severe Grade 3**	**Life Threatening Grade 4**
Fever (oral temp)	< 100.4°F (< 38.0°C)	100.4 – 101.1°F (38.0 – 38.4°C)	101.2 – 102.0°F (38.5 – 38.9°C)	102.1 – 104°F (39.0 – 40°C)	> 104.0°F (>40°C)
Headache	None	No interference with activity	Repeated use of nonnarcotic pain reliever > 24 hours or some interference with activity	Significant; any use of narcotic pain reliever or prevents daily activity	ER visit or hospitalization
Myalgia	None	Easily tolerated, causing minimal discomfort and does not interfere with everyday activities[a]	Sufficiently discomforting to interfere with everyday activities	Prevents normal everyday activities or requires medical advice	ER visit or hospitalization
Abdominal Pain	None	No interference with activity	Some interference with activity not requiring medical intervention	Prevents daily activity and requires medical intervention	ER visit or hospitalization
Anorexia	None	No interference with activity	Some interference with activity not requiring medical intervention	Prevents daily activity and requires medical intervention	ER visit or hospitalization
Nausea/ Vomiting	None	No interference with activity or 1 to 2 episodes/24 hours	Some interference with activity or >2 episodes/24 hours	Prevents daily activity, requires outpatient IV hydration	ER visit or hospitalization for hypotensive shock
Diarrhea	None	2 to 3 loose stools or < 400 gms/24 hours	4–5 stools or 400 to 800 gms/24 hours	6 or more watery stools or > 800gms/24 hours or requires outpatient IV hydration	ER visit or hospitalization
Malaise/ Fatigue	None	No interference with activity	Some interference with activity	Significant; prevents daily activity	ER visit or hospitalization

[a] Everyday activities include attendance at work, school and usual habits of the participants.

Appendix 2 – Grading of Laboratory Abnormalities (Serum Chemistry)

Serum[a]	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)
Sodium – Hyponatremia mEq/L	132 to 134	130 to 131	125 to 129	< 125
Sodium – Hypernatremia mEq/L	144 to 145	146 to 147	148 to 150	> 150
Potassium – Hyperkalemia mEq/L	5.1 to 5.2	5.3 to 5.4	5.5 to 5.6	> 5.6
Potassium – Hypokalemia mEq/L	3.5 to 3.6	3.3 to 3.4	3.1 to 3.2	< 3.1
Glucose – Hypoglycemia mg/dL	65 to 69	55 to 64	45 to 54	< 45
Glucose – Hyperglycemia				
Fasting – mg/dL	100 to 110	111 to 125	>125	Insulin requirements or hyperosmolar coma
Random – mg/dL	110 to 125	126 to 200	>200	
BUN mg/dL	23 to 26	27 to 31	> 31	Requires dialysis
Creatinine – mg/dL	1.5 to 1.7	1.8 to 2.0	2.1 to 2.5	> 2.5 or requires dialysis
Calcium – hypocalcemia mg/dL	8.0 to 8.4	7.5 to 7.9	7.0 to 7.4	< 7.0
Calcium – hypercalcemia mg/dL	10.5 to 11.0	11.1 to 11.5	11.6 to 12.0	> 12.0
Magnesium – hypomagnesemia mg/dL	1.3 to 1.5	1.1 to 1.2	0.9 to 1.0	< 0.9
Phosphorous – hypophosphatemia mg/dL	2.3 to 2.5	2.0 to 2.2	1.6 to 1.9	< 1.6
CPK – mg/dL	1.25 to 1.5 x ULN	1.6 to 3.0 x ULN	3.1 to 10 x ULN	> 10 x ULN
Albumin – Hypoalbuminemia g/dL	2.8 to 3.1	2.5 to 2.7	< 2.5	--
Total Protein – Hypoproteinemia g/dL	5.5 to 6.0	5.0 to 5.4	< 5.0	--
Alkaline phosphate – increase by factor	1.1 to 2.0 x ULN	2.1 to 3.0 x ULN	3.1 to 10 x ULN	> 10 x ULN
Liver Function Tests – ALT, AST increase by factor	1.1 to 2.5 x ULN	2.6 to 5.0 x ULN	5.1 to 10 x ULN	> 10 x ULN
Bilirubin – when accompanied by any increase in Liver Function Test increase by factor	1.1 to 1.25 x ULN	1.26 to1.5 x ULN	1.51 to 1.75 x ULN	> 1.75 x ULN
Bilirubin – when Liver Function Test is normal; increase by factor	1.1 to 1.5 x ULN	1.6 to 2.0 x ULN	2.0 to 3.0 x ULN	> 3.0 x ULN
Cholesterol	201 – 210	211 – 225	> 226	---
Pancreatic enzymes – amylase, lipase	1.1 to 1.5 x ULN	1.6 to 2.0 x ULN	2.1 to 5.0 x ULN	> 5.0 x ULN

ULN = upper limit of normal

[a] The laboratory values provided in the tables serve as guidelines and are dependent upon institutional normal parameters. Institutional normal reference ranges should be provided to demonstrate that they are appropriate.

Appendix 3 – Grading of Laboratory Abnormalities (Hematology)

Hematology[a]	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)
Hemoglobin (Female), gm/dL	11.0 to 12.0	9.5 to 10.9	8.0 to 9.4	< 8.0
Hemoglobin (Female) change from baseline value, gm/dL	Any decrease to 1.5	1.6 to 2.0	2.1 to 5.0	> 5.0
Hemoglobin (Male), gm/dL	12.5 to 13.5	10.5 to 12.4	8.5 to 10.4	< 8.5
Hemoglobin (Male) change from baseline value, gm/dL	Any decrease to 1.5	1.6 to 2.0	2.1 to 5.0	> 5.0
WBC increase, cell/mm^3	10,800 to 15,000	15,001 to 20,000	20,001 to 25,000	> 25,000
WBC decrease, cell/mm^3	2,500 to 3,500	1,500 to 2,499	1,000 to 1,499	< 1,000
Lymphocytes decrease, cell/mm^3	750 to 1,000	500 to 749	250 to 499	< 250
Neutrophils decrease, cell/mm^3	1,500 to 2,000	1,000 to 1,499	500 to 999	< 500
Eosinophils, cell/mm^3	650 to 1500	1501 to 5000	> 5000	Hypereosinophilic
Platelets Decreased, cell/mm^3	125,000 to 140,000	100,000 to 124,000	25,000 to 99,000	< 25,000
PT, increase by factor	1.0 to 1.10 x ULN	1.11 to 1.20 x ULN	1.21 to 1.25 x ULN	> 1.25 ULN
PTT, increase by factor	1.0 to 1.2 x ULN	1.21 to 1.4 x ULN	1.41 to 1.5 x ULN	> 1.5 x ULN
Fibrinogen increase, mg/dL	400 to 500	501 to 600	> 600	--
Fibrinogen decrease, mg/dL	150 to 200	125 to 149	100 to 124	< 100 or associated with gross bleeding or DIC

DIC = disseminated intravascular coagulation; PT = prothrombin time; PTT = partial thromboplastin time; ULN = upper limit of normal; WBC = white blood cell

[a] The laboratory values provided in the tables serve as guidelines and are dependent upon institutional normal parameters. Institutional normal reference ranges should be provided to demonstrate that they are appropriate.

Appendix 4 – Grading of Laboratory Abnormalities (Urinalysis)

Urine*	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)
Protein	Trace	1+	2+	Hospitalization or dialysis
Glucose	Trace	1+	2+	Hospitalization for hyperglycemia
Blood (microscopic), red blood cells per high power field	1 to 10	11 to 50	> 50 and/or gross blood	Hospitalization or packed red blood cells transfusion

[a]The laboratory values provided in the tables serve as guidelines and are dependent upon institutional normal parameters. Institutional normal reference ranges should be provided to demonstrate that they are appropriate.