Protocol Number: VXA-NVV-201

Official Title: A Phase 2b Double-blinded, Randomized, Placebo-Controlled, Human Norovirus GI.1 (Norwalk Virus Inoculum) Challenge Study Following Administration of an Oral, Single-dose Norovirus Vaccine expressing GI.1 VP1 and dsRNA Adjuvant to Protect Against Norovirus Gastroenteritis (NVG) in Healthy Adult Volunteers

NCT Number: NCT05212168

Document Date: 25 May 2023

Clinical Study Protocol

Title	A Phase 2b Double-Blinded, Randomized, Placebo-Controlled, Human Norovirus GI.1 (Norwalk Virus Inoculum) Challenge Study Following Administration of an Oral, Single-dose Norovirus Vaccine expressing GI.1 VP1 and dsRNA adjuvant to Protect Against Norovirus Gastroenteritis (NVG) in Healthy Adult Volunteers
Protocol Number	VXA-NVV-201
IND Number	16796
Product Names	Norovirus GI.1 Norwalk VP1 Vaccine, Oral E1-/E3-Deleted Replication Defective Recombinant Adenovirus 5 with dsRNA Adjuvant (VXA-G1.1-NN) Challenge strain: Norovirus GI.1 (Norwalk Virus Inoculum Lot 001-09NV and Sublot 2)
Study Sponsor	Vaxart, Inc. 170 Harbor Way, Suite 300 South San Francisco, CA 94080
Sponsor Contact	[redacted] Ph: [redacted] Email: [redacted]
Sponsor Medical Monitor	[redacted] Ph: [redacted] Email: [redacted] [redacted] Ph: [redacted]; Email: [redacted]
Clinical Site	[redacted]
Clinical Investigator	Dr. Youngjun David Kim Phone: [redacted]
Protocol Version/Date	Amendment 5, 25 May 2023, Version 6.0
Prior Version	Amendment 4, 07 February 2023, Version 5.0 Amendment 3, 23 June 2022, Version 4.0 Amendment 2, 07 January 2022, Version 3.0 Amendment 1, 01 December 2021, Version 2.0 Original, 28 October 2018, Version 1.0

SIGNATURE PAGE/STATEMENT OF COMPLIANCE

Title: A Phase 2b Double-Blinded, Randomized, Placebo-Controlled, Human Norovirus GI.1 (Norwalk Virus Inoculum) Challenge Study Following Administration of an Oral, Single-dose Norovirus Vaccine expressing GI.1 VP1 and dsRNA adjuvant to Protect Against Norovirus Gastroenteritis (NVG) in Healthy Adult Volunteers

Protocol No: VXA-NVV-201 (Ver. 6.0, Amendment 5)

Vaxart, Inc. [redacted]

6/8/2023
Date

The trial will be conducted in accordance with the International Conference on Harmonisation (ICH) E6 and the Code of Federal Regulations on the Protection of Human Subjects (45 CFR Part 46). Except where necessary to eliminate an immediate hazard(s) to the trial subjects, the Principal Investigator will assure that no deviation from, or changes to the protocol will take place without prior agreement from the sponsor and documented approval from the Institutional Review Board (IRB) and/or Independent Ethics Committee (IEC). All personnel involved in the conduct of this study have completed Human Subjects Protection Training.

I agree to ensure that all staff members involved in the conduct of this study are informed about their obligations in meeting the above commitments.

Principal Investigator: _____ _____
Signature Date

Name: _____
(Print)

Site & Address:
[redacted]

TABLE OF CONTENTS:

SIGNATURE PAGE/STATEMENT OF COMPLIANCE 2
LIST OF ABBREVIATIONS AND ACRONYMS 8
CLINICAL PROTOCOL SYNOPSIS 10
Primary Endpoint 19
Secondary Endpoints 19
1 INTRODUCTION: BACKGROUND INFORMATION AND SCIENTIFIC RATIONALE 22
1.1 Background 22
1.2 Norovirus vaccine: General Properties & Pharmacodynamics 24
1.2.1 VXA-G1.1-NN Product Description 24
1.3 Summary of Nonclinical Studies 24
1.4 Summary of Clinical Studies with Vaxart Oral Vaccine Platform 25
1.4.1 Norovirus Phase 1 Study VXA-G11-101 25
1.4.2 Norovirus Phase 1 Dose Optimization Study VXA-G11-102 28
1.4.3 Clinical Study VXA-NVV-103 with VXA-G1.1-NN and VXA-G2.4-NS 30
Summary of Safety 31
Summary of Immunogenicity Analysis 32
1.5 Norovirus Human Challenge Model 33
1.6 NV Challenge Dose Titration Study and Dose Justification 36
1.7 Summary of Clinical Experience with Vaxart Oral Vaccine Platform 38
1.8 Rationale for GI.1 Human Challenge Study (VXA-NVV-201) 39
1.9 Potential Risks and Benefits 39
1.9.1 Vaccine Risks 39
1.9.2 Norovirus Challenge Risks 40
1.9.3 Risk of Blood Draw 40
1.9.4 Known Potential Benefits 40
2 HYPOTHESIS, OBJECTIVES AND PURPOSE 40
2.1 Study Hypothesis 40
2.2 Study Objectives 40
2.2.1 Primary Objective 40
2.2.2 Secondary Objectives 40
2.2.3 Exploratory Objectives 41
3 STUDY DESIGN AND ENDPOINTS 41
3.1 Description of Study Design 41

3.2 Study Endpoints....42
3.2.1 Primary Endpoint....42
3.2.2 Secondary Endpoints....42
3.2.3 Safety Endpoints....42
3.2.4 Immunogenicity Endpoints....42
4 STUDY ELIGIBILITY, ENROLLMENT AND WITHDRAWAL....43
4.1 Subject Inclusion Criteria....43
4.2 Subject Exclusion Criteria....44
4.3 Withdrawal from the Study....46
4.3.1 Reasons for Withdrawal or Termination....46
4.3.2 Handling of Subject Withdrawals or Termination....47
5 STUDY DRUG....48
5.1 Study Drug(s) and Control Description....48
5.1.1 Drug Product Packaging and Labeling....48
5.1.2 Drug Product Storage and Stability....48
5.1.3 Drug Product Dosage Preparation and Administration....49
5.1.4 Study Drug Accountability....49
6 NV CHALLENGE INOCULUM....50
6.1 Formulation, Packaging, and Labeling of NV Challenge Virus....50
6.2 Storage and Stability of NV Challenge Virus....50
6.3 Preparation and Administration of NV Challenge Virus....51
7 STUDY PROCEDURES AND SCHEDULE....51
7.1 Pre-Screening Period (Day -90 to Screening)....51
7.2 Visit 00: Screening Visit (Study Day -45 to -1)....52
7.3 Visit 01: Study Day 1, Study Day Administration....52
7.4 Visit 02: Study Day 8....53
7.5 Visit 03: Study Day 28, 1 day prior to challenge [+3 days for visit window and +30 days window for repeat Visit 03]....53
7.6 Visit 04: Study Day 29 (Challenge Day)....55
7.6.1 Pre-challenge Assessments....55
7.6.2 Viral Challenge Eligibility....55
7.6.3 Viral Challenge Administration....56
7.6.4 Visits 04 – 08: Post-Challenge Observation Period (Day 29 – Day 33 [+ 3 Days]) 56
7.7 Visit 09: Study Day 36 (7 Days post challenge)....57
7.8 Visit 10: Study Day 57 (28 Days post challenge ± 3 days)....58

7.9 Visit 11 (Day 120) – Visit 14 (Day 300) Safety Follow-up Contacts 58
7.10 Visit 15: Study Day 365 (337 days post challenge + 1 week) End of Study Contact 58
7.11 Early Termination 59
7.11.1 Early Termination (Day 1 – Day 28; Vaccine Phase) 59
7.11.2 Early Termination (–ay 29 - Day 57; Challenge Phase) 59
7.11.3 Early Termination (Day 58 to Day 365 – Safety Follow-Up Period) 59
7.12 Unscheduled Visit(s) 60
7.13 Immunogenicity Assessments 60
7.14 Assessment of Clinical Illness 60
7.15 Norwalk Virus Infection 60
7.16 Microbiological Assessments 61
8 MANAGEMENT OF EXPECTED NOROVIRUS ILLNESS 61
8.1 Clinical Evaluation 61
8.2 Study Definitions 61
8.3 Measurement of Diarrhea and Vomitus 61
8.4 Management of Fluid Loss 62
8.5 Indications for Concomitant Medications 62
8.6 Prohibited and Controlled Medications/Treatments 63
9 ASSESSMENT OF SAFETY 63
9.1 Definition of Adverse Event 63
9.1 Solicited Symptom Reactogenicity Table 63
9.1.2 Grading of Severity of an Adverse Event 65
9.1.3 Relationship to Challenge Virus 65
9.1.4 Relationship to Study Vaccination 65
9.2 Definition of Serious Adverse Event 66
9.3 Definition Adverse Events of Special Interest (AESI) 66
9.4 Definition of New Onset of Chronic Illness (NOCI) 66
9.5 Study Halting Rules 67
9.6 Safety Oversight 67
9.7 Safety Monitoring Committee (SMC) 67
10 CLINICAL MONITORING 68
11 STATISTICAL CONSIDERATIONS 68
11.1 Study Hypothesis 68
11.2 Sample Size Determination 69
11.3 Treatment Assignment Procedures 69

11.3.1 Randomization Procedures 69
11.3.2 Blinding Procedures 70
11.4 Final Analysis Plan 70
11.5 Missing Values and Outliers 70
11.6 Interim Analysis 71
11.7 Exploratory Analysis 71
12 SOURCE DOCUMENTS AND ACCESS TO SOURCE DATA/DOCUMENTS .. 72
12.1 Source Documentation 72
12.2 Data Management 72
13 QUALITY ASSURANCE AND QUALITY CONTROL 72
14 ETHICAL CONSIDERATIONS (AND INFORMED CONSENT) 73
14.1 Ethical Standard 73
14.2 Institutional Review Board 73
14.3 Informed Consent Process 73
14.4 Exclusion of Women, Minorities, and Children (Special Populations) 73
14.5 Subject Confidentiality 74
15 REFERENCES 74
APPENDIX A: SCHEDULE OF EVENTS 77
APPENDIX B: CHALLENGE VIRUS PREPARATION 78
APPENDIX C: GRADING OF SIGNS AND SYMPTOMS OF ACUTE GASTROENTERITIS 82
APPENDIX D: MODIFIED VESIKARI SCALE (17 POINTS) 83
APPENDIX E: LIST OF ADVERSE EVENTS OF SPECIAL INTEREST (AESIs) .. 84
APPENDIX F: Preparation of NV Challenge Inoculum 87
APPENDIX G: SUMMARY OF CHANGES TO PROTOCOL 91

TABLES AND FIGURES

Table 1: VXA-G1.1-NN Product Description 24
Table 2: Subject Enrollment in Study VXA-G11-101 26
Table 3: Solicited Symptoms (Solicited TEAEs in Study VXA-G1.1-101) 26
Table 4: Geometric Mean Titer (GMT), GM Fold Rise (GMFR) and Statistical Significance for Leb and H1 BT_{50} assay in Study VXA-G11-101 27
Table 5: Treatment Groups and Dosing Regimens in Study VXA-G11-102 28
Table 6: Solicited Adverse Events in Study VXA-G11-102 29
Table 7: Comparison of BT_{50} Antibody Results Among Groups in the VXA-G11-101 and VXA-G11-102 Trials 29
Table 8: Design of Study VXA-NVV-103 30

Table 9: Summary of Solicited Symptoms of Reactogenicity (Study VXA-NVV-103)……31
Table 10: Norwalk Virus Infection and Illness Rates after Challenge with NV 8fIIa (Atmar Study) . 33
Table 11: Dose response for Norwalk strain 8fIIa and 8fIIb (Teunis Study)..... 34
Table 12: Summary of Norovirus Challenge Studies – C. Moe's Lab; Emory University..... 34
Table 13: Symptoms Reported After Challenge with Norwalk Strain 8fIIb..... 35
Table 14: Description of illness after challenge with two different doses of Norwalk virus (Study VXA-G11-201.1)..... 36
Table 15: Summary of the correlation between acute gastroenteritis and NV positive by cohort….. 37
Table 16: Characterization of diarrhea in subjects with acute gastroenteritis and NV positivity by cohort (by mean)……...37
Table 17: Clinical Studies Completed Using Vaxart Oral Ad5 Vaccine Platform, 2011-2017 38
Table 18: Study Design and Vaccine Groups (Study VXA-NVV-201)..... 42
Table 19: Active Product Description (VXA-G1.1-NN) 48
Table 20: SOLICITED SYMPTOM REACTOGENICITY TABLE …… 60
Table 21: Power Calculations ……..69

Figure 1: VP1-Specific Antibody Secreting Cells Measured 7 Days After Immunization (Study VXA-G1.1-101)..... 27
Figure 2: Overall IgA ASC Response Rate After Either One or Two Doses (Study VXA-G1.1-102)..... 30

LIST OF ABBREVIATIONS AND ACRONYMS

AE	Adverse Event
AESI	Adverse Events of Special Interest
ASC	Antibody secreting cell
BT_{50}	blocking titer fifty assay
BUN	blood urea nitrogen
CBC	Complete blood count
CFR	Code of Federal Regulations
cGMP	Current Good Manufacturing Practice
CMP	Clinical Monitoring Plan
CoA	Certificate of Analysis
CRF	Case Report Form
CRO	Contract Research Organization
CyTOF	Cytometry by Time of Flight
DP	Drug Product
dsRNA	Double-stranded ribonucleic acid
ECG	Electrocardiogram
e-Diary	Electronic diary
ELISA	Enzyme-linked Immunosorbent Assay
FDA	Food and Drug Administration
GI	Norovirus Genogroup I
GII	Norovirus Genogroup II
GC	Genome copies
GCP	Good Clinical Practice
GERD	Gastroesophageal reflux disease
GLP	Good Laboratory Practice
GMFR	Geometric mean fold rise
GMP	Good Manufacturing Practice
GMT	Geometric mean titer
GRAS	Generally recognized as safe
HBGA	Histo-blood group antigen
HbsAg	Hepatitis B surface antigen
HCV	Hepatitis C virus
HDPE	High-density polyethylene
HIPAA	Health Insurance Portability and Accountability Act
HIV	Human Immunodeficiency Virus
ICF	Informed Consent Form
ICH	International Conference on Harmonisation
IEC	Independent or Institutional Ethics Committee
IgA	Immunoglobulin A
IgG	Immunoglobulin G
IND	Investigational New Drug Application
IRB	Institutional Review Board
IU	Infectious unit(s)
IV	Intravenous
MedDRA	Medical Dictionary for Regulatory Activities
MLF	Mucosal lining fluid
MM	Medical Monitor
Nab	Neutralizing antibody
NOCI	New Onset of Chronic Illness

NoV	Norovirus
NV	Norwalk Virus
NVG	Norovirus gastroenteritis
OHRP	Office for Human Research Protections
PBMC	Peripheral Blood Mononuclear Cells
PI	Principal Investigator
PF4	Platelet Factor 4 antibody ELISA
QA	Quality Assurance
QC	Quality Control
rAd5	recombinant Ad5
qRT-PCR	Quantitative Reverse Transcriptase Polymerase Chain Reaction
SAE	Serious Adverse Event/Serious Adverse Experience
SAM	Synthetic absorption matrix
SigA	Secretory IgA
SMC	Safety Monitoring Committee
SOP	Standard Operating Procedure
TTS	Thrombolytic and thrombocytopenic syndrome
US	United States
USDA	United States Department of Agriculture
US EPA	United States Environmental Protection Agency
VLP	Virus-like Particle
VP1	Viral protein 1, major capsid or surface protein of viruses
WHO-DD	World Health Organization Drug Dictionary

CLINICAL PROTOCOL SYNOPSIS

Study Title	A Phase 2b Double-Blinded, Randomized, Placebo-Controlled, Human Norovirus GI.1 (Norwalk Virus Inoculum) Challenge Study Following Administration of an Oral, Single-dose Norovirus Vaccine expressing GI.1 VP1 and dsRNA adjuvant to Protect Against Norovirus Gastroenteritis (NVG) in Healthy Adult Volunteers
Protocol Number	**VXA-NVV-201**
Sponsor	Vaxart, Inc.
IND Number	16796
Trial Phase	Phase 2b
Study Population	Approximately 170 healthy male and female adult volunteers age 18 to 49 years inclusive with blood type O or A and who are confirmed H type-1 antigen secretory positive (approximately 170 subjects will be randomized and vaccinated to ensure that at least 140 subjects are available to participate in the viral challenge 28 days post-vaccination)
Investigational Site	1 qualified US site (with isolation unit) • [redacted]
Investigational Product	Active Vaccine: • Norovirus GI.1 Norwalk VP1 Vaccine (VXA-G1.1-NN), an Oral E1/E3-Deleted Replication-Defective Recombinant Adenovirus serotype 5 with double-stranded ribonucleic acid (dsRNA) Adjuvant. The vaccine vector encodes for a full-length VP1 gene from Norwalk virus (NV). The adjuvant is a short hairpin RNA, expressed as a 21 nucleotide sequence (GAAACGA TATGGGCTGAATAC) as a tandem sequence in forward and reverse orientations separated by 6 nucleotides that comprise the loop of the RNA. The final drug product (DP) is formulated into enteric-coated tablet. • Dose: $1x10^{11}$ IU±0.5 log Placebo Control: • Oral tablets similar in appearance and number to active vaccine tablets Multiple tablets of study drug will be dispensed to allow delivery of the intended vaccine dose ($1x10^{11}$ IU). A matching number of placebo tablets will be dispensed to maintain the study blinding.
Viral Challenge Inoculum	Norovirus GI.1 (Norwalk Virus Inoculum Lot 001-09NV and Sublot 2, IND 14697) • Dose: $1x10^{6}$ Genomic Copies (GC). A dose which allows 50% - 65% infectivity in the healthy adult population (per NV infection rate observed in the GI.1 viral titration study (Mateo, et al; 2019)
Study Hypothesis	Norovirus vaccine (VXA-G1.1-NN) will protect against Norovirus Gastroenteritis (NVG) related to norovirus (NoV) infection in the challenge model

Study Objectives	**Primary Objective** • Determine the clinical efficacy of VXA-G1.1-NN compared to placebo, to protect against NVG caused by the Norwalk strain challenge inoculum. **Secondary Objectives** • Safety and tolerability of VXA-G1.1-NN oral vaccine • Determine the ability of VXA-G1.1-NN to modify disease severity (defined in **Appendices C and D**) compared to placebo • Determine the quantity and duration of norovirus shedding by qRT PCR • Evaluate the VP1 specific IgA ASC, HBGA blocking antibody, and VP1 specific serum IgG responses to VXA-G1.1-NN **Exploratory Objectives** • Determine correlation of immunogenicity parameters with clinical outcome • Further evaluate immunogenicity of VXA-G1.1-NN
Study Definitions	NVG is a composite endpoint defined as meeting one or more of the following definitions for both Acute Gastroenteritis and NV Infection. **Acute Gastroenteritis** (defined as meeting any one of the three categories listed below): • Diarrhea: o ≥ 3 loose or liquid stools produced in any 24-hour period, or o > 400g of loose or liquid stools produced in any 24-hour period • Vomiting: ≥ 2 vomiting episodes in any 24-hour period, or • Diarrhea and vomiting: o One vomiting episode plus any loose or liquid stool in any 24-hour period, or o One vomiting episode plus ≥ 2 of the following events in any 24-hour period: ▪ nausea ▪ fever (oral temperature ≥ 37.6°C) ▪ abdominal cramps or pains ▪ abdominal gurgling or bloating ▪ myalgia See **Appendix C** for grading scales for signs and symptoms of acute gastroenteritis. **Norwalk Virus Infection:** • NV infection as detected by qRT-PCR, > 1 positive post-challenge stool or emesis sample through day 8 post-challenge
Study Design	This is a Phase 2b randomized, double-blind, placebo-controlled vaccination and challenge study to assess the protective efficacy of the Norovirus vaccine (VXA-G1.1-NN). Approximately 170 healthy adults will be randomized in a 1:1 ratio to receive one oral dose of vaccine or placebo. • Arm 1: VXA-G1.1-NN oral vaccine tablets [$1x10^{11}$ IU±0.5 log]; N=85 • Arm 2: Placebo tablets similar in appearance and number to active vaccine tablets; N=85

	To accommodate the limited size of the isolation unit that will be utilized for the challenge and post-challenge sequestration period, subjects will move through the study (enrollment, vaccination and challenge) sequentially in 14 to 16 cohorts. Subjects will be randomized in cohorts of 10 to 12 each. Approximately 28 days post-vaccination, each cohort will be admitted to the isolation ward and challenged with the NV GI.1 Norwalk challenge strain. After challenge, subjects will be monitored for signs and symptoms of AGE at least twice daily from Day 29 to discharge. Stool and emesis will be collected, and illness will be assessed using a modified Vesikari scale. Excretion of NV (shedding) will be monitored in stool and emesis. NV illness lasts 2-4 days and is self-limited. At 4 days post challenge (Day 33) asymptomatic subject will be discharged from the isolation ward and will be followed in a series of outpatient visits and telephone calls. Symptomatic subjects may be kept in the isolation ward for up to an additional 3 days. Approximately 170 subjects will be dosed in the vaccination phase to ensure at least 140 subjects (approximately 70 VXA-G1.1-NN vaccine and 70 placebo) are available to participate in the challenge phase. **Investigational Drug Product** / **Approximate No. of Subjects** **Vaccination** — **170** VXA-G1.1-NN oral vaccine tablets [$1x10^{11}$ IU±0.5 log] — 85 Placebo tablets (identical to vaccine) — 85 **Challenge (**Norwalk GI.1 Virus Inoculum) — **140** VXA-G1.1-NN oral vaccine tablets [$1x10^{11}$ IU±0.5 log] — 70 Placebo tablets (identical to vaccine) — 70 An independent Safety Monitoring Committee (SMC) will convene at pre-defined intervals during the norovirus challenge period, and also *ad hoc* as needed during the vaccination and challenge periods, to oversee the safety of the study.
Study Visits	The following study visits and remote contacts will be conducted during the study (also see **Appendix A**: Schedule of Events): **Vaccination Phase:** • Pre-Screening Period (Days -90 to Screening) may be utilized for purposes of ascertaining subjects' H type-1 antigen secretory status and blood type • Screening Period (Days -45 to -1) • Day 1 Visit (Baseline assessments; day of randomization and vaccination) • Day 8 Visit (safety and evaluation of immune response) • Day 28 (evaluation of immune response; 1 day prior to challenge, start inpatient stay) **Challenge Phase:**

	• Day 29 (viral challenge, sequestration) • Days 30 to 33 (sequestration – discharge; +3 days and evaluation of immune response and safety assessment) • Day 36 Visit (evaluation of immune response and safety assessment) • Day 57 Visit (evaluation of immune response and safety assessment and end of active period) **Safety Follow-Up:** • Day 120, Day 180, Day 240 and Day 300 (follow-up contact) • Day 365 (follow-up phone call): Study completion
Eligibility Criteria	**<u>Inclusion Criteria:</u>** 1. Male or female between the ages of 18 – 49 years, inclusive 2. Able to give written informed consent 3. Healthy[1], as determined by the principal investigator (PI) or PI in consultation with the research monitor and Sponsor *[1]No clinically significant health concerns or medical illness), as determined by medical history, physical examination, vital signs, electrocardiogram (ECG), and clinical laboratories (complete blood count [CBC], chemistry and urinalysis)* 4. Comprehension of the study requirements with ability and willingness to complete all assessments and comply with confinement period post viral challenge, and all scheduled visits and contacts 5. Confirmed blood type (A or O) 6. Demonstrated to be H type-1 antigen secretor positive (by saliva test) 7. Body mass index between 17 and 35 kg/m^2, inclusive, at Screening 8. Female participants must have a negative pregnancy test at pre-vaccination and pre-challenge <u>and</u> fulfill one of the following[2] *[2]Criteria:* *a. At least one year post-menopausal;* *b. Surgically sterile;* *c. Use of oral, implantable, transdermal, intravaginal or injectable contraceptives for 30 days prior to immunization and until 60 days after challenge;* *i. A reliable form of contraception must be approved by the Investigator (eg, double barrier method, Depo-Provera, intrauterine device, Norplant, oral contraceptives, contraceptive patches, vaginal ring contraceptive)* *d. Not be sexually active (abstinent) or in a same sex relationship (must be discussed with site staff and documented)* 9. Male subjects must agree not to father a child or donate sperm, as well as to use contraception/barrier (a male condom) or be abstinent from heterosexual intercourse, from vaccination through the active period (Day 57)

Exclusion Criteria:

1. Administration/use of any investigational drug or device 30 days prior to vaccination through the active period (Day 57)
2. Administration of any licensed vaccine within 30 days prior to vaccination or planned use of the above stated during the active period (through Day 57)
3. Presence of a significant medical condition[3] which in the opinion of the investigator precludes participation in the study.
   [3]For example, psychiatric conditions, or gastrointestinal disease, such as peptic ulcer, symptoms or evidence of active gastritis or gastroesophageal reflux disease, inflammatory bowel disease, alcohol or illicit drug abuse/dependency, or other laboratory abnormalities
4. Laboratory values outside the range of normal for platelet counts and the following coagulation tests: PT/INR, aPTT and fibrinogen
5. Any of the following history or conditions that may lead to higher risk of clotting events and/or thrombocytopenia:
   a. Family or personal history of bleeding or thrombosis
   b. History of heparin-related thrombotic events, and/or receiving heparin treatments
   c. History of autoimmune or inflammatory disease
   d. Presence of any of the following conditions known to increase risk of thrombosis within 6 months prior to screening:
      - Recent surgery other than removal/biopsy of cutaneous lesions
      - Immobility (confined to bed or wheelchair for 3 or more successive days)
      - Head trauma with loss of consciousness or documented brain injury
      - Receipt of anticoagulants for prophylaxis of thrombosis
      - Recent clinically significant infection
6. Any one of the following ECG findings[4] within 45 days prior to vaccination:
   [4]Exclusionary ECG findings:
   a. QTc F (interval duration > 450 msec (male) or > 470 msec (female)
   b. QRS interval greater than 120 msec
   c. PR interval greater than 220 msec
   d. Clinically significant ST-T wave changes or pathologic Q waves
7. History of cancer or cancer treatment within past 3 years (excluding basal cell or squamous cell carcinomas)

	8. Presence of immunosuppression or medical condition possibly associated with impaired immune responsiveness, including diabetes mellitus or angioedema 9. Donation or use of blood or blood products within 30 days prior to vaccination through the active period (Day 57) 10. Diagnosed bleeding disorder or significant bruising or bleeding difficulties that could make blood draws problematic 11. Any condition that resulted in the absence or removal of the spleen 12. Evidence of confirmed infection with human immunodeficiency virus (HIV), hepatitis B surface antigen (HbsAg) or hepatitis C virus (HCV) with confirmatory assays 13. Abnormal stool pattern (fewer than 3 bowel movements per week or more than 3 per day) 14. History of irritable bowel disease or inflammatory digestive or gastrointestinal condition[5] that could affect the distribution / safety evaluation of an orally administered vaccine[6] [5]*Such conditions may include but are not limited to:* *a. Esophageal Motility Disorder* *b. Malignancy* *c. Malabsorption (e.g. Celiac disease, gluten intolerance)* *d. Pancreaticobiliary disorders* *e. Irritable bowel syndrome* *f. Inflammatory Bowel Disease* *g. Surgical Resection with the exception of appendectomy or a minor resection that is deemed acceptable by investigator and sponsor* *h. Gastroesophageal reflux disease (GERD)* *i. Hiatal Hernia* *j. Peptic Ulcer* *(History of cholecystectomy is not exclusionary)* [6] *targeting the mucosa of the small intestine* 15. Use of proton pump inhibitors, H2 blockers or antacids within 7 days prior to vaccination through the active period (Day 57) 16. Use of antibiotics within 30 days prior to vaccination through the active period (Day 57)[7] [7]*Note: use of a brief (≤ 10 days) course of oral or topical antibiotic for minor URI, UTI, dental work, or skin infection allowed within the screening period, but must be completed 7 days prior to first vaccination* 17. Use of medication known to affect the immune function (e.g. systemic corticosteroids and others) within 14 days prior to vaccination through the active period (Day 57) 18. Regular use of nonsteroidal anti-inflammatory drugs within 7 days prior to vaccination through the active period (Day 57)

19. Use of over-the-counter probiotics or antidiarrheals within 7 days prior to vaccination through the active period (Day 57)
20. Evidence of recent (within 2 months of vaccination) or of current nonbacterial gastroenteritis suggestive of NV infection [vomiting or unformed or watery stools (> 2 during a 24-hour period)]
21. Any gastroenteritis within the past 2 weeks prior to vaccination
22. Acute disease within 72 hours prior to vaccination[8]
    [8]*Defined as the presence of a moderate or severe illness with or without fever (as determined by the Investigator through medical history and physical examination). (Assessment may be repeated during screening period)*
23. Presence of a fever ≥ 38ºC measured orally at baseline
24. History if hematochezia (blood in stool) or melena (black stool)
25. Any significant hospitalization within the last year which in the opinion of the investigator or sponsor could interfere with study participation
26. History of serious reactions to any vaccination such as anaphylaxis, respiratory problems, Guillain-Barre syndrome, hives or abdominal pain
27. History of a hypersensitivity or allergic reaction to any component of the investigational vaccine or placebo[9]. Subjects with known fish allergies should be excluded
    [9] *including but not limited to fish gelatin*
28. History of drug, alcohol or chemical abuse within 1 year prior to vaccination
29. Positive test for drugs of abuse or alcohol at screening, vaccination baseline and pre-challenge.[10]
    [10]*except for previous marijuana use; concurrent or ongoing use of marijuana during the active study period*
30. Consistent/habitual smoking within 2 months prior to vaccination (defined as smoking ≥ 1 pack of cigarettes a day). Smoking is not permitted during the inpatient stay
31. Other conditions[11] that would jeopardize the safety or rights of a subject or interfere with the evaluation of the study
    [11] *in the clinical judgment of the investigator*

<u>Social/Occupational:</u>

32. Living with or having daily contact with children ≤ 5 years old or women known to be pregnant or nursing[12]
    [12]*This includes significant contact at home, school, day-care, or equivalent facilities*
33. Living with or having daily contact with elderly persons ≥ 70 years of age or infirmed, diapered individuals, persons with disabilities or incontinence[13]
    [13]*This includes at work or visits to nursing homes and day-care or equivalent facilities*

	34. Employment in the food service industry such as restaurant or cafeteria facilities[14] [14]*Specifically, this includes persons whose employment requires food handling and processing in the 4 weeks following viral challenge* 35. Health-care workers with patient contact expected in the 4 weeks following viral challenge 36. Expected contact[15] with immunocompromised persons[16] in the 4 weeks following viral challenge [15] *via employment or at home* [16] *HIV-positive, receiving immunosuppressive medications such as oral steroids, anti-neoplastic agents* 37. Presence of household members who have received the Ad4 or Ad7 vaccines within 2 months prior to vaccination 38. Employment as an airline flight attendant or cruise ship crew, scheduled to work in the 4 weeks following challenge 39. Persons planning to live in a confined environment (eg, a cruise, camp, etc.) in the 4 weeks following viral challenge
Study Duration	Each subject's participation will last approximately 1 year post successful screening and enrollment • 90 days study pre-screening • 45 days study screening • 28 days vaccination phase • 28 days viral challenge and follow-up • 365 days total safety follow-up post vaccination
Randomization	Subjects will be randomized in a 1:1 ratio to receive either oral VXA-G1.1-NN vaccine or placebo. A randomization list will be prepared allocating subject identification numbers to the study treatment group. The randomization scheme will be provided to the unblinded research pharmacist at the clinical site.
Blinding	Investigators, clinical staff and study subjects will remain blinded until completion of the challenge phase (through Day 57) and validation of the clinical data. The Sponsor's clinical team and laboratory personnel will also be blinded to subject treatment through Day 57 analyses. Each bottle of multi-dose DP/placebo tablets will be labeled with an open label. The unblinded research pharmacist will use the randomization list to prepare the dose of DP/placebo tablets. All DP/placebo preparation and administration will be performed per study specific procedures detailed in the Study Pharmacy Manual.
Safety, Immunogenicity and Microbiological Assessments	**<u>Safety Assessments:</u>** <u>General:</u> • SAEs, AESIs & NOCIs will be recorded from Baseline (Day 1) through Day 365 <u>Vaccination Period (Day 1 to Day 28):</u> • Solicited symptoms of reactogenicity will be recorded daily from Day 1 post vaccination through Day 8:

	○ Gastrointestinal reactions: nausea, vomiting, diarrhea and abdominal pain ○ Systemic reactogenicity: malaise/fatigue, anorexia, fever, headache and myalgia (muscle pain) • Unsolicited Aes will be recorded from Day 1 (post-vaccination) through Day 28 • 12-lead ECGs at Screening and pre-challenge (Day 28) • Vital signs and physical exams will be collected at Screening, Day 1 (pre-dose), Day 8, and Day 28 (pre-challenge) • Safety laboratory will be performed at Screening, Day 1, Day 8 and Day 28 (pre-challenge) as listed below: ○ CBC: Hemoglobin, Hematocrit, Platelet Count and Complete White Blood Cell Count ○ Serum chemistries: ALT, AST, Total Bilirubin, BUN, Creatinine, Random Glucose, Potassium and Sodium ○ Urinalysis: Glucose, Protein and Hemoglobin • Female participants will have serum/urine pregnancy tests at Screening, Day 1 (pre-dose, if more than 30 days from last test) and Day 28 pre-challenge <u>Challenge Period (Day 29 to Day 57):</u> • Unsolicited Aes will continue to be recorded from challenge (Day 29) through end of active phase (Day 57) • Vital signs will be collected twice a day (am and pm) from challenge (Day 29) through discharge (Day 33 + 3 days) • Physical exams will be performed daily on Days 29 through discharge (Day 33 + 3 days) • Safety laboratory tests will be performed on Day 33. Additional time points may be collected if clinically indicated per the discretion of the Investigator **Immunogenicity Assessments:** <u>Primary Immunoassays</u> • VP1 specific IgA ASC (by ELISpot) • HBGA blocking antibodies (BT_{50}) • VP1 specific serum IgG (by MSD) • VP1 specific serum IgA (by MSD) <u>Exploratory Immunoassays</u> • VP1 specific IgG ASC (by ELISpot) • VP1 specific IgG and IgA memory B cells (by ELISpot) • VP1 specific IgG and IgA from plasmablast cultures (by ELISA) • B cell immunophenotyping (by flow cytometry, CyTOF and/or single cell sequencing) • mucosal VP1 specific IgA (by ELISA or MDS), (fecal, saliva and nasal)

	Blood Typing Blood typing will be measured via blood group antigens (ABO) test **Assessment of Clinical NV Illness (Acute Gastroenteritis):** Following challenge (Day 29) subjects will be monitored for the following signs and symptoms of acute gastroenteritis twice a day unless greater frequency is clinically indicated: o diarrhea, vomiting, nausea, fever, myalgia o abdominal cramps or pain and abdominal gurgling or bloating **Norwalk Virus Infection:** • NV infection as detected by qRT-PCR, > 1 positive post-challenge stool or emesis sample through day 8 post-challenge. **Microbiological Assessments:** During the post-challenge phase (Day 29 – discharge [Day 33 + 3 days]) all stool and emesis specimens will be collected, weighed/measured for volume and tested for NV shedding through discharge. Stool samples will also be graded. A stool sample will also be collected at the Day 36 and Day 57 site visits.
Study Endpoints	**Primary Endpoint** • Occurrence of NVG caused by the challenge norovirus during inpatient challenge period. o NVG is a composite endpoint and is defined as meeting one or more of the definitions for both acute gastroenteritis and NV infection. **Secondary Endpoints** • Number of subjects with acute gastroenteritis during the inpatient challenge period. • Mean score using the Modified Vesikari Scale as a measure of severity of acute gastroenteritis post-challenge at discharge (up to Day 36) • Number of subjects with moderate or severe gastroenteritis (cumulative loose stools ≥ 1000 gms during the inpatient period) • Median duration (number of hours) of acute gastroenteritis among challenged subjects • Number of subjects with norovirus infection up to Day 36 post-challenge (quantitative reverse transcriptase polymerase chain reaction [qRT-PCR] positive in stool, Norwalk virus antigen in stool • Geometric mean genome copies of norovirus shedding by qRT-PCR • Median duration (number of hours) of norovirus infection among challenge subjects • Number of incidences of diarrhea or emesis among challenge subjects

	SAFETY • Vaccine (VXA-G11-N.N) o Solicited symptoms of reactogenicity x 7 days post-vaccination o Unsolicited Aes x 28 days post vaccination o SAEs, AESIs and NOCIs x 12 months post vaccination • Challenge Virus (Norwalk GI.1) o Unsolicited Aes x 28 days post challenge **IMMUNOGENICITY** **Primary** • VP1 specific IgA ASC response against Norwalk at Day 8 compared to placebo • GMT of HBGA blocking antibodies (by BT_{50}) against Norwalk virus at Day 28 compared to placebo • VP1 specific serum IgG response at Day 28 compared to placebo • VP1 specific serum IgA at Day 28 compared to placebo **Exploratory** • Correlations between individual immunogenicity parameters and clinical efficacy • VP1 specific IgG ASC response against Norwalk at Day 8 compared to placebo • VP1 specific fecal IgA at Day 28 compared to placebo • VP1 specific saliva IgA at Day 8 and Day 28 compared to placebo • VP1 specific nasal IgA at Day 8 and Day 28 compared to placebo • Vaccine specific responses for memory IgA and/or IgG in B cells, IgA and IgG in plasmablasts, antibody repertoire sequencing analysis, flow cytometry (including homing and/or immunophenotyping analyses), cytof and/or other assays designed to evaluate cell mediated immunity Additional assessment of serum, fecal, saliva, and mucosal immunity at other time points (including post-challenge) may be performed
Halting Rules	The study will be halted (no new enrollment, vaccination or challenge will be allowed pending a SMC safety review) if any of the events described below are met during the conduct of the study. • Two or more subjects experience a treatment related serious adverse event (SAE) of any grade, or a treatment related grade 3 or 4 AE between study Day 1 and Day 28 (vaccination phase). • Two or more subjects experience a treatment related serious adverse event (SAE) of any grade, or a treatment related grade 3 or 4 unsolicited adverse event within 28 days after challenge. The SMC will provide study oversight throughout the duration of the trial interventional and safety follow-up period (Day 1 through Day 365 post-vaccination). The IRB and CBER (Center for Biologics Evaluation and Research at the US FDA) will be notified if the study is halted for safety concerns.
Statistical Considerations	The study hypothesis is that norovirus vaccine, VXA-G1.1-NN, will protect against norovirus gastroenteritis related to norovirus infection in the challenge model. Protective efficacy (PE) will be estimated by the prevention of

	norovirus gastroenteritis among vaccine recipients compared to placebo recipients in those who were challenged. The primary endpoint of this study is a composite of occurrence of NVG caused by the challenge norovirus during inpatient challenge period and is defined as meeting one or more of the definitions for both acute gastroenteritis and NV infection. An Interim analysis (IA) will take place after approximately 50% of the planned randomized subjects have completed challenge and been discharged from the research unit. This IA will reassess the appropriateness of assumptions used for the primary efficacy endpoint when the trial was designed in a blinded manner to the study team. The IA data will be analyzed in an unblinded manner for the SMC. An exploratory analyses will be performed after approximately 50 subjects of the planned randomized subjects have been vaccinated with the same IP lot and completed the challenge period. The results of the exploratory analyses will not impact the continuation of this study as planned, but rather, inform program development for other trials. Additionally, a blinded interim immunogenicity analysis will be performed on Cohorts 1-9 and shared with the sponsor. The results of the analyses will not impact the continuation of this study as planned, but rather, inform program development for other trials. Please refer to **Section 11** for complete statistical considerations details in the protocol.

1 INTRODUCTION: BACKGROUND INFORMATION AND SCIENTIFIC RATIONALE

1.1 Background

Human noroviruses (NoV) are the leading cause of foodborne gastroenteritis and endemic diarrheal disease in the United States (US) and worldwide (1, 2). Norovirus is highly infectious and is transmitted very efficiently from person to person in semi-enclosed environments, such as nursing homes, cruise ships, hospitals, military, schools and childcare centers (3). The major mode of transmission is by fecal-oral spread, usually via contaminated food or water. Airborne transmission via aerosolization of the virus with vomiting has also been reported (4). The incubation period of norovirus is 10-51 hours. The clinical symptoms of norovirus include a sudden onset of nausea, vomiting, non-bloody diarrhea and abdominal cramps. Fever, headache and body aches have been reported as well. These symptoms usually last 2-4 days. Additionally, asymptomatic infections are estimated to occur in approximately one third of infected people (5). Infections occur year-round but peak in the colder months (November to April in the Northern Hemisphere). All ages are susceptible to infection; however, the elderly seem to be more susceptible. Viral shedding can last several weeks (6-55 days) with a peak of 1 -3 days after onset of illness (6, 7). Viral shedding precedes the onset of illness in up to 30% of infected people (5). Currently no specific therapy exists for norovirus gastroenteritis (NVG). The standard treatment is oral rehydration with fluids and electrolytes (8).

Noroviruses (NoVs) belong to the family Caliciviridae. Caliciviruses are divided into 4 genera: Norovirus, Sapovirus, Vesivirus and Lagovirus. NoVs are genetically diverse viruses. The genus is divided into 6 genogroups. NoV Genogroups I, II and IV are human-transmitted. Genogroup I and II account for the majority of norovirus outbreaks (9). Each genogroup is further divided into genotypes based on the similarity of the amino acid sequence of the major viral capsid protein, VP1. Genogroup I contains 9 different genotypes and Genogroup II contains 22 different genotypes. Norwalk virus, the prototype human norovirus, is classified as a Genogroup I and Genotype 1 (GI.1) virus (2). Since the 1990's, GII.4 viruses have been responsible for more than 80% of all human norovirus infections, but non-GII.4 (Genogroup I and other Genogroup II genotypes) are also circulating in the population. Every 2-4 years, a new GII.4 variant emerges and replaces the previously predominant variant (10, 11). In 2012, a new GII.4 variant was identified in Australia. This GII.4 variant, the Sydney strain is currently the most prevalent norovirus globally. It may have emerged by escape from herd immunity acquired against the previously predominant circulating strain (12, 13). In contrast, GII.17 norovirus has rarely been reported as a major genotype. However, in winter 2014-2015, a new GII.17 variant emerged and caused most of the outbreaks in China and Japan (14). Noroviruses are positive-sense, non-enveloped, single-stranded RNA viruses. The viral genome is 7.4-7.7 kb in length and comprises 3 open reading frames (ORFs) that encode both the structural and non-structural proteins. The ORF1 (~5kb) is in the first two-thirds of the genome and encodes ~200 kD large poly-protein that is cleaved into 6 different non-structural proteins essential for viral replication such as RNA dependent RNA polymerase. ORF2 (~1.6kb) encodes a 57kDa single major capsid protein (VP1). VP1 is comprised of a conserved internal shell domain (S) and protruding domain (P). The P domain is further divided into a P1 stalk sub-domain and a surface-exposed highly variable P2 sub-domain. P2 sub-domain is the major immunogenic and antigenic determinant of the NoV. VP1 forms a dimer and 90 dimers form a T=3, icosahedral viral particle. ORF3 (~0.6kb) encodes a 22kDa minor structural protein (VP2), whose function might package the viral genome into virions and assist capsid assembly. VP1 associates with VP2 at the interior surface (S domain) of the capsid (15, 16).

The expression of VP1 results in spontaneous self-assembly of virus-like particles (VLP) without packaging the viral nucleic acid or proteins. VLP is a non-infectious particle and is morphologically and antigenically identical to the native NoV (17).

Histo-blood group antigens (HBGAs) are putative norovirus binding receptors or attachment factors. HBGAs are complex glycans that are expressed on the surfaces of red blood cells, gut, and respiratory epithelia. Different norovirus species display different HBGA binding profiles. The P2 sub-domain in VP1 appears to be the HBGA interaction site (18).

Because norovirus cannot easily be cultured in tissue culture, classical virus neutralization cannot be directly measured. Instead, the VLP-HBGA blocking assay has been used as a surrogate to measure the ability of antibodies from antisera from infected people to block VLP binding to HBGA (19). The duration of protective immunity upon natural infection is complex. Human challenge studies using Norwalk virus as the challenge virus suggested that short-term immunity to homologous virus develops and persists for up to 6 months. However, a subsequent study showed that longer-term immunity (27-42 months) was not generated after homologous challenge (20, 21). It is not completely clear what immune parameters are critical for protection from challenge, but one possibility is that mucosal Immunoglobulin A (IgA) may be able to hinder virus infection of epithelial cells. Consistent with this, published challenge studies showed that an early induction of mucosal IgA or preexisting mucosal IgA response correlated with protection to Norwalk virus (7, 22). In contrast to the results derived from the challenge studies that long-term immunity can be difficult to generate, the epochal pattern of evolution of pandemic GII.4 strongly suggests the development of herd immunity in the population (12). Some recent mathematical models based on modeling of epidemiological data estimated that protective immunity after natural infection may last 4 to 8 years (23). In summary, these results suggest that natural immunity can develop, with some caveats as to whether long-term sterilizing immunity is possible. Several animal species including humanized mice, gnotobiotic pigs, gnotobiotic calves, and chimpanzees have been challenged with human NoV. However, NoV infection of non-natural hosts generated variable clinical outcomes from asymptomatic to mild diarrhea, and limited success has been achieved to reveal all aspects of human infection (24).

There are currently no licensed vaccines for norovirus. Experimental norovirus vaccines composed of VLPs expressing VP1 have been tested in mice and humans delivered orally and intranasally (25-29). Takeda Pharmaceutical Company (previously Ligocyte) conducted two short-term efficacy studies. Intranasal administration of Norwalk VLP vaccine was shown to reduce Norwalk virus-associated acute gastroenteritis (69% vs 37%) and Norwalk virus infection (82% vs 61%) following challenge with homologous Norwalk virus (30). A subsequent study was conducted with intramuscular bivalent vaccine (Norwalk virus VLP and a consensus VLP derived from three GII.4 NoV strains). Healthy participants were challenged by GII.4 NoV (Cin strain 2003, Farmington Hill Strain variant). The vaccines were well tolerated and immunogenic. However, the incidence of GII.4 NoV-associated acute gastroenteritis (33% vs 26%) and GII.4 NoV infection (62.5% vs 54%) were not significantly reduced although severity of illness was reduced (31). These GII.4 results seem rather unexpected since the intramuscular bivalent vaccine generated much higher total serum Immunoglobulin G (IgG) titers and blocking titers than the intranasal mucosal vaccine. One possible explanation is that the mucosal vaccine might provide better mucosal antibody responses, and thereby better local protection against norovirus infection (27).

Norovirus is an enteric pathogen that infects the epithelial cells of the small intestine. Importantly, Vaxart's platform is based on oral delivery to the intestinal mucosa, which is analogous to the natural route of norovirus infection and entry. In theory, mucosal immune responses local to the site of delivery should be more robust than mucosal responses generated by a more distally administered vaccine. Human and animal experience with Vaxart's orally administered platform has demonstrated that substantial transgene specific intestinal IgA responses can be generated in addition to systemic IgG responses to the expressed antigen (influenza HA and norovirus VP1). These platform attributes may allow for better protection against norovirus infection than an injected protein-based vaccine.

1.2 Norovirus vaccine: General Properties & Pharmacodynamics

1.2.1 VXA-G1.1-NN Product Description

VXA-G1.1-NN product description is summarized in **Table 1.**

Table 1: VXA-G1.1-NN Product Description

Generic name	VXA-G1.1-NN
Chemical name	Ad-CMV-VP1(Norwalk virus)-BGH-CMV-luc dsRNA-SPA
Genome size	33,949 bp dsRNA genome
Viral particle size	80-100 nm

VXA-G1.1-NN is E1/E3-deleted, replication-incompetent, adenovirus 5 vaccine vectors designed for use as a vaccine for prevention of NoV infection. The vaccine vector encodes for a full-length VP1 gene of Norwalk virus (VXA-G1.1-NN vaccine). In addition to the transgene cassette, a second hCMVie promoter is also present in the vaccine construct which is used to express an RNA sequence that acts as an adjuvant. The adjuvant is a short hairpin RNA expressed off a promoter such that only target cells in the intestine that express antigen will also express the adjuvant. This is likely to result in a tight association of antigen with adjuvant *in vivo*.

1.3 Summary of Nonclinical Studies

Vaxart has conducted preclinical studies to determine the immunogenic potential of two NoV vaccine candidates, VXA-G1.1-NN (VP1 gene of Norwalk virus) in mice and VXA-G2.4-NS (VP1 gene of Sydney virus) in mice, ferrets and NHPs (Cynomolgus monkeys). These studies showed that vaccination with VXA-G1.1-NN and VXA-G2.4-NS reliably elicited substantial and durable systemic serum IgG and intestinal (fecal) IgA responses in test animals and elicited serum-blocking antibody titers (blocking titer fifty assay [BT50]). A unique immune response elicited by oral vaccination is the induction of antibodies derived from intestinal B cells. After oral vaccination or enteric infection, B cells residing in the lamina propria (underneath the gut epithelial cells) predominantly produce dimeric/polymeric antibodies of the IgA isotype. After binding to polymeric immunoglobulin receptor (also known as secretory component), the dimeric form of IgA antibodies travels across the gut epithelial cells into the lumen through facilitated transport (transcytosis), leaving a secretory component attached to dimeric IgA. The resulting molecule is known as secretory IgA (SigA). SigA serves as an additional external barrier to block enteric infection. SigAs are eventually flushed out of the system and can be detected in fecal samples. Given that the route of immunization is the same as the route of norovirus infection, it is reasonable to postulate that Vaxart's VXA-G1.1-NN and VXA-G2.4-NS vaccines may enable higher degrees of localized protection against infection when compared to a parenteral immunization method. This is supported by data on the immune responses generated in mice orally immunized with VXA-G1.1-NN when compared to mice intramuscularly injected with GI.1 VLPs. Vaxart's oral vaccine generated significantly higher fecal IgA when compared to the injectable formulation.

As described above, Vaxart's norovirus VP1 vaccines (VXA-G1.1-NN and VXA-G2.4-NS) use the same replication-defective viral vector backbone and adjuvant RNA sequence as the company's early oral vaccine candidates for pandemic influenza (the A/Indonesia/05/2005 (H5N1) vaccine referred to as ND1.1 and for seasonal influenza (the A/California/ 04/2009 (H1N1) vaccine referred to as VXA-A1.1. Because the only difference between the vaccines is the antigen gene [NoV VP1 in VXA-G1.1-NN and VXA-G2.4-NS versus A/California/04/2009 (H1N1) in VXA-A1.1 and A/Indonesia/05/2005 (H5N1) in ND1.1], the preclinical studies from ND1.1 and VXA-A1.1 are relevant to and support the

clinical development of Vaxart's NoV vaccines (VXA-G1.1-NN and VXA-G2.4-NS) for prevention of norovirus disease.

Preclinical studies with ND1.1 showed that no vector distributed beyond the target tissues in the gut and that multiple oral immunizations over a 3-month period were well tolerated. The ND1.1 test material used in the toxicology studies and for most of the other preclinical and clinical studies, was manufactured using the same process that is used to produce the Good Manufacturing Practice (GMP) material for the proposed clinical trial. VXA-G1.1-NN and VXA-G2.4-NS vaccines is formulated as enteric-coated tablets for oral administration in clinical studies, similar to VXA-A1.1 clinical drug product (DP) lots. It is important to note that in several nonclinical studies reported within this section a liquid formulation of the ND1.1 vaccine was delivered orally by gavage or via endoscopic instillation into the lumen of the duodenum because of technical limitations that prevented the use of enteric-coated tablets in the test animal species.

The role of the adjuvant in the oral vaccine platform construct was studied with the seasonal vaccine candidate VXA-A1.1. The primary objective of the H1N1 immunogenicity study was to determine if a double-stranded ribonucleic acid (dsRNA) adjuvant could augment immune responses to the transgene expressed after transduction with the recombinant Ad5-based (rAd5) vector. Study results demonstrated that a co-expressed dsRNA adjuvant improved the immune responses to an orally administered antigen and that the immune responses generated could elicit protective immunity to H1 influenza.

In another immunogenicity study (Study No. WCB045), mice were immunized by oral gavage with varying doses of recombinant adenoviral vector expressing HA and dsRNA (VXA-A1.1) or one expressing HA but not the dsRNA adjuvant (rAd-HA). Three weeks post immunization the group treated with the adjuvanted vector demonstrated a significant anti-HA titer (1.0×10^4 geometric mean titers [GMT]) with all animals having a detectable titer, whereas the unadjuvanted group failed to generate a detectable response in any animals. The immune responses were significantly higher for the adjuvant compared to the non-adjuvant groups. These data indicate that the adjuvant enhances both the time of onset and the magnitude of the immune response.

1.4 Summary of Clinical Studies with Vaxart Oral Vaccine Platform

Summary information on trials completed with Vaxart's norovirus vaccine candidates is presented below. For additional information refer to the Investigator's Brochure.

1.4.1 Norovirus Phase 1 Study VXA-G11-101

This was a single-site, randomized, double-blind, placebo-controlled clinical trial of VXA-G11-NN oral norovirus vaccine to determine safety and immunogenicity. The tablet vaccine is comprised of a non-replicating adenovirus-based vector expressing the VP1 gene from the GI.1 norovirus strain and a dsRNA adjuvant.

Between July and September of 2016, 152 subjects were screened, and 66 subjects were enrolled and dosed. Sixty five of 66 subjects completed safety and immunogenicity assessments through the active phase (Day 29); one subject in the placebo group withdrew before Day 7. The dose groups are described in **Table 2**.

Table 2: Subject Enrollment in Study VXA-G11-101

Vaccine group	Dose	Number of oral doses	Number of subjects
VXA-G1.1-NN	low dose – $1x10^{10}$ IU	1	23
VXA-G1.1-NN	high dose – $1x10^{11}$ IU	1	23
Placebo		1	20

Safety Results

The vaccine was well-tolerated, with no dose-limiting toxicities. Adverse events (AEs) were mild or moderate. All solicited AEs reported in this trial (n=46) were grade 1 or 2 in severity with the majority being mild events (44 grade 1, and 2 grades 2 events). The percentage of subjects with any solicited symptoms was similar between test and placebo treatments (**Table 3**). In the first 7 days following study drug administration, 35 study subjects had at least one solicited AEs reported with 25/46 (54%) subjects in the VXA-G1.1-NN vaccine groups and 10/20 (50%) subjects in the placebo groups. Diarrhea and headache were the most common solicited symptoms following VXA-G1.1-NN administration, both reported by 15 (33%) subjects in the treated groups. Headache and nausea were reported evenly across treatments, including placebo. The only solicited symptom demonstrating a statistically significant difference from placebo was diarrhea (P=0.0275), reported by 11 subjects in the high dose group. Nine of the 11 subjects reported mild severity diarrhea, while 2 subjects reported moderate severity episodes following the high dose vaccine. Onset of diarrhea (verbatim term "loose stools") ranged from Day 1 to Day 6 following vaccine administration, and most episodes resolved within 1 day. At no point did any of the loose stool symptoms impact normal activity such as work or school, and none required treatment with anti-diarrheal medications or rehydration therapy. There were no SAEs, AESIs or NOCIs reported in this study through Day 365.

Table 3: Solicited Symptoms (Solicited TEAEs in Study VXA-G11-101)

Adverse Events	Placebo (n=20)	Low dose (n=23)	High dose (n=23)
Number of Subjects with Solicited Symptom TEAEs	10 (50%)	11 (48%)	14 (61%)
Diarrhea	3 (15%)	4 (17%)	11 (48%)
Abdominal Pain	2 (10%)	5 (22%)	0
Nausea	4 (20%)	4 (17%)	3 (13%)
Malaise	2 (10%)	1 (4%)	3 (13%)
Headache	8 (40%)	8 (35%)	7 (30%)

Immunogenicity Results

Antibody responses to Norovirus VP1 were assessed primarily by BT_{50} assay. Titers were measured by using either Leb or H1 synthetic glycan as the coating antigen. Using the Leb assay, 14/23 (61%) subjects had a 2-fold rise in the low dose group, and 18/23 (78%) had a 2-fold rise or greater in the high dose group. One subject in the placebo group had a greater than 2-fold rise. On day 28, the GMT by the Leb assay for low dose vaccine group was 59.0 (95% CI 33.0-105.4), a 2.3-fold geometric mean fold rise (GMFR) over the initial GMT of 26.2 (95% CI 16.6-41.2) at baseline. The GMT for the high dose vaccine group was 98.5 (95% CI 64.4-150.7), a 3.8-fold GMFR over the initial GMT of 25.8 (95% CI 18.3-36.2) at baseline (32). Similar observations were made for titers measured with H1. The high dose group had significantly increased titers compared to placebo on Day 28 by either Leb (P=0.0003) or H1 (P=0.001) BT_{50} assay **Table 4**.

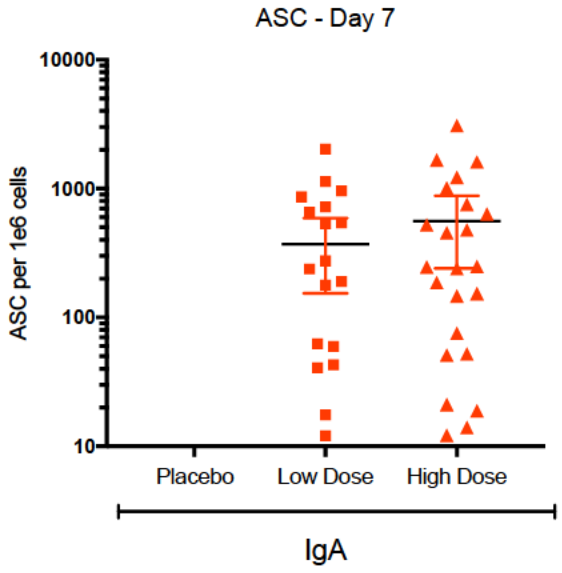
Table 4: Geometric Mean Titer (GMT), GM Fold Rise (GMFR) and Statistical Significance for Leb and H1 BT_{50} assay in Study VXA-G11-101

HBGAs	Leb[1]				H1			
Group	D0 GMT	D28 GMT	GMFR	p value[2]	D0 GMT	D28 GMT	GMFR	p value
Low	26.2	59	2.3	0.046	22.8	50	2.2	0.045
High	25.8	98.5	3.8	0.0003	22.5	69.7	3.1	0.0013
Placebo	24.6	27.4	1.1	reference	22	23.7	1.1	reference

[1]Histo-Blood Group Antigens
[2]Significance by Mann-Whitney for vaccine vs placebo; overall significance by Kruskal Wallis test.

Antigen-specific IgA Antibody Secreting Cell responses. The ability of the vaccine to induce antigen specific antibody producing B cells in peripheral blood was measured by antibody secreting cell (ASC) assay with particular focus on IgA ASC. In the low dose group, 16/23 (70%) subjects had positive IgA ASC responses 7 days after vaccination, and in the high dose group, 19/23 (83%) subjects had positive responses. Background ASCs were generally negligible on day 0. For the high dose group, an average of 561 IgA ASCs per 1 x 10^6 peripheral blood mononuclear cells (PBMCs) were detected on day 7, **Figure 1**. For the low dose group, an average of 372 IgA ASCs was found on day 7. The placebo group had no significant response. The vaccine treated groups were significantly different than placebo in terms of their ability to elicit an IgA ASC response at day 7 ($P<0.0001$ by Kruskal-Wallis). A positive ASC response was defined as a count after vaccination of at least 3 SDs above the mean pre-vaccination count and at least 13 spots/well/106 PBMCs (IgG) and 23 spots/well/10^6 PBMCs (IgA).

Figure 1: VP1-Specific Antibody Secreting Cells Measured 7 Days After Immunization (Study VXA-G1.1-101)



Additional immunology analysis. For a pathogen such as norovirus that invades through the intestinal epithelial, local (intestinal) protection and memory IgA responses are believed to be important. These responses were assessed by measuring the increase in the norovirus VP1 specific responses in fecal samples pre and post immunization, measuring the homing potential of the activated B cells post immunization, and direct assessment of the antigen specific memory B cell expansion induced in the

peripheral blood post immunization. In terms of fecal IgA, in the high dose group 9/19 (47%) subjects had a 4-fold or greater IgA responses at Day 28, and 9/21 (43%) subjects had 4-fold or greater response at Day 180; the average fold increases in the specific IgA/total IgA ratio were 17.2 and 9.7, respectively. Fecal IgA responses in the low dose group were similar to those of the high dose group, with 7/20 (35%) and 5/16 (31%) showing 4-fold increases on Days 28, and 180, respectively. The average fold increase in specific IgA for the low dose group was 36.2 on Day 28 and 5.6 on Day 180.

Flow cytometry was used to assess the intestinal cell homing potential post immunization. Substantial α4β7hi (the intestinal mucosal) expression was observed on the activated B cells 7 days post immunization with up to 25% of all B cells having upregulated the intestinal homing receptor. In terms of increases in memory IgA, the GMFR for IgA was 15.3 for the high dose group suggesting substantial expansion of the antigen specific memory cell pool. Similarly, in the low dose group, a GMFR of 7.4 for IgA was observed at Day 7.

Summary

The vaccine was well-tolerated, with no dose-limiting toxicities. AEs were mild or moderate. The primary immunological endpoint (increase in BT_{50} titers) was met in the high dose group (P=0.0003) with 78% showing ≥ 2-fold rise after a single immunization. Vaccine recipients also developed mucosally-primed VP1-specific circulating ASC, IgA+ memory B cells expressing gut homing receptor α4β7) and fecal IgA, indicating robust and local responses clinically relevant to prevent norovirus infection.

1.4.2 Norovirus Phase 1 Dose Optimization Study VXA-G11-102

This was an open-label dose optimization trial of VXA-G1.1-NN oral norovirus vaccine to further determine safety and immunogenicity with differing dosing regimens. Fifteen subjects were enrolled in 1 of 4 cohorts for a total enrollment of 60 subjects. The dose and dose regimen are described in **Table 5.**

Table 5: Treatment Groups and Dosing Regimens in Study VXA-G11-102

Group	Vaccine group	Dose	Number of oral doses	Dosing days	Number of subjects
1	VXA-G1.1-NN	low dose – $1x10^{10}$ IU	2	0, 7	15
2	VXA-G1.1-NN	low dose – $1x10^{10}$ IU	3	0, 2, 4	15
3	VXA-G1.1-NN	low dose – $1x10^{10}$ IU	2	0, 28	15
4	VXA-G1.1-NN	high dose – $1x10^{11}$ IU	2	0, 28	15

Safety

The oral vaccine was well tolerated. There were no SAEs, AESIs or NOCIs reported in this study through Day 365. The most frequently reported symptoms are showed in **Table 6**. Diarrhea, which was a frequently reported symptom in the high dose group in the initial norovirus vaccine study (VXA-G11-101), was not commonly reported in this study.

Table 6: Solicited Adverse Events in Study VXA-G11-102

Solicited Treatment Emergent Adverse Events (TEAEs)	Group 1 (n=15)	Group 2 (n=15)	Group 3 (n=15)	Group 4 (n=15)
Number of Subjects with Solicited TEAEs	5 (33%)	8 (53%)	11 (73%)	3 (20%)
Diarrhea	0	1 (7%)	5 (33%)	1 (7%)
Abdominal Pain	1 (7%)	1 (7%)	3 (20%)	1 (7%)
Nausea	1 (7%)	2 (13%)	2 (13%)	0
Malaise	2 (13%)	0	2 (13%)	1 (7%)
Feeling Hot	0	1 (7%)	0	0
Headache	4 (27%)	7 (47%)	9 (60%)	1 (7%)

Immune Response

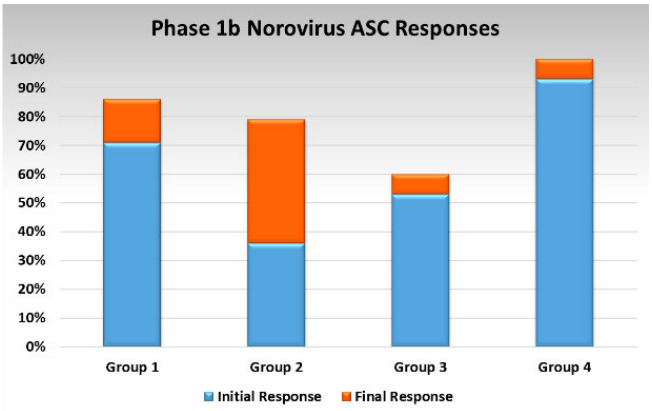
The objective of the study was to compare schedules and dosing for the ability to elicit immune responses. BT_{50} titers were assessed at multiple times points, given that multiple doses were given. In the high dose group, 12 of 15 subjects had a 2-fold or greater increase in BT_{50} titers after the first dose and 14 of 15 subjects (92%) had a 2-fold or greater increase in BT_{50} titers after 2 doses. The GMT titer rose from 21.3 on day to 85.1 on day 28 for a 3.8 GMFR. The GMT at Day 56 were measured to be 75.8, a GMFR of 3.6 over the baseline values**. Table 7** compares the 101 and 102 high dose results. Other groups given lower doses of vaccine had lower response rates. Groups 1 and 3 had higher increases in the titers compared to Group 2, although this is not statistically significant. Using an ANCOVA model, the significance in the different groups to increase the GMFR, was found to be P=0.0008, 0.1224, 0.0004, and <0.0001 for groups 1 through 4 respectively at Day 56. This means all groups had statistically significant increases in the GMT except for group 2, which had a more modest increase in the titers.

Table 7: Comparison of BT_{50} Antibody Results Among Groups in the VXA-G11-101 and VXA-G11-102 Trials

Study	Dose group	Dosing (day)	D0 GMT	D28 GMT	GMFR	D56 GMT	GMFR D56
101	Placebo	1	24.6	28.1	1.1	N/A	N/A
101	High dose	1	25.8	97.9	3.8	N/A	N/A
102	High dose	1 and 28	21.3	85.1	4	75.8	3.6

An additional immunological analysis was performed by comparing the IgA ASC responders between groups, Figure **2**. The high dose group had 14 out of 15 subjects respond to the vaccine, with an average IgA ASC count of 698 per 1 x 10^6 PBMCs. Following a second dose, the subject that didn't respond the first time had a significant increase in ASC counts so all 15 subjects (100%) were able to elicit an ASC response following two doses. The low dose groups were compared by examining the overall response rate, since the dosing and the analysis were performed at different intermediate timepoints. Group A had the highest overall response rate where 12/14 subjects (86%) were able to induce meaningful ASC responses after 1 or 2 doses. Slightly lower responders were observed in group B, where only a few subjects had a response after the first dose, but more subjects responded after additional vaccine doses. Group C had the most variable responses of any group. The average number of spots was 839 per 1 x 10^6 cells after the first dose, but this was the result of several subjects having extremely high numbers of spots (3 subjects had greater than 1500 per 1 x 10^6), mixed with many subjects that didn't respond at all. A positive ASC was defined as higher than 8 spots per 10^6 PBMCs and at least 3 spots/well/3x10^5 PBMCs (IgA).

Figure 2: Overall IgA ASC Response Rate After Either One or Two Doses (Study VXA-G11-102)



1.4.3 Clinical Study VXA-NVV-103 with VXA-G1.1-NN and VXA-G2.4-NS

Overall Study Design and Plan

Protocol VXA-NVV-103 Entitled "A Phase 1b, Multi-Center, Randomized, Double-Blind, Placebo-Controlled Safety and Immunogenicity Study of Adenoviral-vector Based Oral Norovirus Vaccines Expressing GI.1 or GII.4 VP1 with Monovalent or Bivalent Dosing in Healthy Adult Volunteers" is a multi-center, randomized, double-blind, placebo-controlled study to assess the safety and immunogenicity of monovalent GI.1, monovalent GII.4, bivalent GI.1/GII.4 norovirus vaccines, or placebo at differing dose levels with varying dosing schedules.

After a sentinel lead-in group (n=5) dosed with GII.4 vaccine, subjects were randomized in a 1:1:2:1 manner to one of four treatment arms (**Table 8**) and followed for primary safety and immunogenicity for 29 days and for long-term safety though Day 365.

A total of 80 subjects entered the study and were randomized to study treatment. A total of 77 subjects completed the study. Three subjects in the Bivalent GII.4/GI.1 group discontinued the study (1 subject withdrew consent on Day 8, and 2 subjects were lost to follow up).

Table 8: Design of Study VXA-NVV-103

Group	Vaccine	Dose (IU)	No. of Subjects
1	Monovalent GII.4	$5x10^{10}$	5 sentinels 15 randomized
2	Monovalent GI.1	$5x10^{10}$	15 randomized
3	Bivalent GII.4 and GI.1	$5x10^{10}$ x 2	30 randomized
4	Placebo	0	15 randomized
		Total	**80**

IU = International Units

Summary of Safety

There were no deaths, AESIs, NOCIs, or subject discontinuations due to TEAEs in this study through the duration of the Active Period (Day 29). Overall, 14 subjects reported a TEAE. The incidence of TEAEs was highest in the placebo group (33.3%) compared with the Monovalent GI.1 group (26.7%), Monovalent GII.4 group (15.0%), and the Bivalent GII.4/GI.I group (6.7%). Incidence of study vaccine related TEAEs was highest in the Monovalent GI.1 group (20%) compared with the placebo group (13.3%), Monovalent GII.4 group (5.0%), and the Bivalent GII.4/GI.I group (3.3%). One subject in the Monovalent GII.4 group reported an SAE of Hyperemesis Gravidarum.

The highest number of possibly related TEAEs (20%) were reported in 3 subjects in the Monovalent GI.1 group. There was one probably related TEAE (6.7%) in the placebo group. Most subjects reported solicited symptoms that were mild in intensity. Five subjects reported solicited symptoms of Grade 3 severity. Three subjects (1 each in the Monovalent GII.4 group, Monovalent GI.1 group, and Bivalent GII.4/GI.I group) had ≥ 1 severe solicited symptom.

The incidence of diarrhea was higher across the vaccine treated subjects compared to placebo. The incidence of nausea and headache was highest in Bivalent GII.4/GI.I group compared to other groups. The incidence of malaise/fatigue was higher across the vaccine treated subjects compared to placebo. Myalgia and fever were reported only in the vaccine treated subjects (**Table 9**).

No treatment-related trends were observed regarding clinical laboratory evaluations. Several laboratory TEAEs were reported; however, the investigator considered a mild TEAE of decreased blood phosphorus in 1 subject in the Bivalent GII.4/GI.1 group as possibly related to study vaccine. No trends were observed regarding vital signs or physical examinations with respect to subject safety.

Table 9: Summary of Solicited Symptoms of Reactogenicity (Study VXA-NVV-103)

Grades 1-3	**Monovalent GII.4 (N=20) n (%)**	**Monovalent GI.1 (N=15) n (%)**	**Bivalent GII.4/GI.I (N=30) n (%)**	**Placebo (N=15) n (%)**
Number of Subjects with at least 1 symptom of Grades 1-3	9	8	16	4
Diarrhea	4 (20.0)	3 (20.0)	6 (20.0)	1 (6.7)
Nausea	3 (15.0)	1 (6.7)	6 (20.0)	2 (13.3)
Vomiting	1 (5.0)	0 (0.0)	2 (6.7)	0 (0.0)
Abdominal Pain	3 (15.0)	1 (6.7)	4 (13.3)	2 (13.3)
Malaise/Fatigue	4 (20.0)	4 (26.7)	6 (20.0)	1 (6.7)
Myalgia (Muscle Pain)	2 (10.0)	2 (13.3)	2 (6.7)	0 (0.0)
Anorexia	0 (0.0)	0 (0.0)	1 (3.3)	0 (0.0)
Headache	2 (10.0)	2 (13.3)	7 (23.3)	2 (13.3)
Fever	1 (5.0)	2 (13.3)	1 (3.3)	0 (0.0)
Temperature				
< 99 F	3 (15.0)	3 (20.0)	9 (30.0)	2 (13.3)
99 to 99.9 F	5 (25.0)	3 (20.0)	6 (20.0)	2 (13.3)
>= 100 F	1 (5.0)	2 (13.3)	1 (3.3)	0 (0.0)

The temperature summarizes the highest temperature recorded for 7 days following each vaccine.

Summary of Immunogenicity Analysis

A summary of immunogenicity results in the Active Period (Day 29) is provided below:

- Statistically significant increases in Serum GI.1 BT_{50} GMT were seen in the Monovalent GI.1 group (p=0.0008) and Bivalent GII.4/GI.1 group (p=0.0084) compared with placebo. No significant differences in the GMT were seen between the Monovalent GI.1 and Bivalent GII.4/GI.1 groups (p=0.2772).
- On Day 29, statistically significant increases in Serum GII.4 BT_{50} GMT were seen in the Monovalent GII.4 group (p= 0.0017) and Bivalent GII.4/GI.1 group (p<0.0001) compared with placebo. No significant differences in the GMT were seen between the Monovalent GII.4 and Bivalent GII.4/GI.1 groups (p=0.2320).
- On Day 8, statistically significant increases in the average counts of GI.1 IgA were seen in the Monovalent GI.1 group (p<0.0001), Bivalent GII.4/GI.1 group (p<0.0001), and Monovalent GII.4 group (p=0.0001) compared with placebo. No significant differences in the average counts of GI.1 IgA were seen between the Monovalent GI.1 and Bivalent GII.4/GI.1 groups (p=0.6013).
- On Day 8, statistically significant increases in the average counts of GII.4 IgA were seen in the Bivalent GII.4/GI.1 group (p<0.0001), Monovalent GII.4 group (p<0.0001) and Monovalent GI.1 group (p=0.0080) compared with placebo. No significant differences in the average counts of GI.1 IgA were seen between the Monovalent GII.4 and Bivalent GII.4/GI.1 groups (p=0.6079).
- On Day 29, statistically significant increases in the GMT of VP1 specific serum GI.1 IgG were seen in the Monovalent GI.1 group (p=0.0010) and the Bivalent GII.4/GI.1 group (p=0.0092) compared with placebo. No significant differences in the GMT were seen between the Monovalent GI.1 and Bivalent GII.4/GI.1 groups (p=0.1293).
- On Day 29, statistically significant increases in the GMT of VP1 specific serum GII.4 IgG were seen in the Bivalent GII.4/GI.1 group (p<0.0001) and the Monovalent GII.4 group (p=0.0001) compared with placebo. No significant differences in the GMT were seen between the Monovalent GII.4 and Bivalent GII.4/GI.1 groups (p=0.3932).
- On Day 8, statistically significant increases in the average counts of ASC GI.1 IgG were seen in the Monovalent GII.4 group (p=0.0002), Monovalent GI.1 group (p=0.0019), and the Bivalent GII.4/GI.1 group (p<0.0001) compared with placebo. No significant differences in the average counts of ASC GI.1 IgG were seen between the Monovalent GI.1 and Bivalent GII.4/GI.1 groups (p=0.4172).
- On Day 8, statistically significant increases in the average counts of ASC GII.4 IgG were seen in the Bivalent GII.4/GI.1 group (p<0.0001) and Monovalent GII.4 group (p<0.0001) compared with placebo. No significant differences in the average counts of ASC GII.4 IgG were seen between the Monovalent GII.4 and Bivalent GII.4/GI.1 groups (p=0.2694).
- On Day 29, statistically significant increases in the GMT of VP1 specific serum GI.1 IgA were seen in the Monovalent GI.1 group (p=0.0391) compared with placebo. No significant differences in the GMT of VP1 specific serum GI.1 IgA were seen between the Monovalent GI.1 and Bivalent GII.4/GI.1 groups (p=0.6184).
- On Day 29, statistically significant increases in the GMT of VP1 specific serum GII.4 IgA were seen in the Bivalent GII.4/GI.1 group (p=0.0002) and the Monovalent GII.4 group (p=0.0018) compared with placebo. No significant differences in the GMT of VP1 specific serum GII.4 IgA were seen between the Monovalent GII.4 and Bivalent GII.4/GI.1 groups (p=0.6295).

1.5 Norovirus Human Challenge Model

In a study by Atmar et al. subjects received a challenge dose of 8fIIa in a multisite trial to evaluate a NV vaccine (6). Subjects were challenged orally with 48 reverse-transcriptase-polymerase-chain-reaction (RT-PCR) units of NV (approximately 10 times the amount of inoculum required to infect 50% of persons to whom it is administered) (6). After virus inoculation, study subjects were assessed for symptoms and signs of gastroenteritis at least twice daily until discharge (the minimum length of stay was 96 hours [4 days]), and stool samples were collected to identify NV.

Viral gastroenteritis was defined as evidence of NV infection and 1 or more of the following symptoms during the inpatient stay: (1) production of >200 grams of watery feces in a 24-hour period; (2) vomiting plus production of < 200 grams of watery feces on the same or consecutive days; or (3) vomiting plus at least 1 constitutional symptom (abdominal cramps or pain, nausea, bloating, loose feces, fever > 37.6°C, myalgia) on the same or consecutive days. NV infection was defined as evidence of fecal virus shedding by real-time RT-PCR or antigen detection or seroresponse (4-fold or greater antibody increase) in total NV serum antibody from the pre-challenge to the day 30 post-challenge time points.

In the per-protocol analysis NV associated gastroenteritis occurred in 69% of the placebo recipients, **Table 10**. The Vesikari disease severity score was 6.7 among subjects in the placebo group with NVG. The onset occurred 33.8 hours after challenge and the illness had a median duration of 29.4 hours (range 0 to 108 hours).

Table 10: Norwalk Virus Infection and Illness Rates after Challenge with NV 8fIIa (Atmar Study)

Per-protocol analysis	Vaccine	Placebo
	N = 38	N = 39
	no. (%)	
NV infection and illness	14 (37)	27 (69)
NV infection by RT-PCR	23 (61)	32 (82)
Vesikari score		
All subjects	3.6	5.5
NV infection	5.0	6.1
NV infection and illness	6.4	6.7
Duration of NV illness		
Median in hours	14.5	29.4
Range in hours	0 to 85.5	0 to 108
Time to onset in hours	35.9	33.8
From Atmar et al. (30)		

After the Atmar study, NV strain 8fIIa was passaged in a human and the resultant strain was designated 8fIIb. Teunis et al. compared the infection and illness rates for 8fIIa and 8fIIb (3). In contrast to Atmar who measured the dose RT-PCR units, Teunis measured dose as genomic copies. According to Atmar, 1 RT-PCR-detectable unit is equivalent to about 400 NV genome copies (GCs). Based on this conversion factor, the dose of 48 RT-PCR units used in the vaccine trial in **Table 11** is equivalent to about $1.92x10^4$ GCs. However, Liu determined in titration studies that 1 RT-PCR-detectable unit is equivalent to about 12-38 NV GCs (33).

Teunis et al. exposed 53 subjects to doses of 8fIIa ranging from $3x10^1$ to $3x10^8$ GCs (3). A dose response was observed with a peak illness rate in 6/8 (75%) of subjects at a dose of $3x10^5$ GC **Table 11**. Doses above this level did not increase the illness rate. The daughter strain 8fIIb was given at doses ranging from $7x10^5$ to $2x\ 10^7$. Nine (33%) of 27 subjects became ill at those doses. Although the attack rate was not statistically different for 8fIIa and 8fIIb, there was a decrease in illness rate with 8fIIb.

Table 11: Dose response for Norwalk strain 8fIIa and 8fIIb (Teunis Study)

	Secretor positive status				
8fIIa dose (GC)	No. of subjects	No. Infected	% Infected	No. Ill	% Ill
$3x10^1$	8	0	0	0	0.0
$3x10^2$	9	0	0	0	0.0
$3x10^3$	9	3	33.3	1	11.1
$3x10^4$	3	2	66.7	1	33.3
$3x10^5$	8	7	87.5	6	75.0
$3x10^6$	7	3	42.9	1	14.3
$3x10^7$	3	2	66.7	2	66.7
$3x10^8$	6	5	83.3	4	66.7
Total	53	22	41.5	15	28.3
8fIIb dose					
$7 x 10^5$	8	3	37.5	2	25.0
$7 x 10^6$	18	14	77.8	7	38.9
$2 x 10^7$	1	1	100.0	NA	
Total	27	18	66.7	9	33.3

Source: adapted from Teunis (3)

NV strain 8fIIb was used as a human challenge strain in 2 other studies (**Table 12**) conducted in Dr. Christine Moe's lab at Emory University. At a dose of 10^7 GC, 10 out of 13 (76.9%) subjects became infected. In all subjects the dose was well tolerated and no SAEs were reported (34, 35).

Table 12: Summary of Norovirus Challenge Studies – C. Moe's Lab; Emory University

Trial	Sponsor	Year	Challenge strain	Dose (GC)	No. subj. challenged	No. infected	% infected
NV persistence in water	US EPA	2006	8FIIb	10^7	13	10	76.9
NV persistence in oysters	USDA	2009	8FIIb	unknown	51	16	31.4

US EPA = United States Environmental Protection Agency
USDA = United State Department of Agriculture

The most common symptoms reported by Leon et al. (34) among 13 infected subjects NV 8fIIb were low grade fever, nausea, vomiting and diarrhea **Table 13**.

Table 13: Symptoms Reported After Challenge with Norwalk Strain 8fIIb

Symptom	No. (%) of subjects with symptom n=13
Chills	3 (23)
Cramping	3 (23)
Diarrhea	6 (46)
Fatigue	5 (38)
Fever	12 (92)
Headache	2 (15)
Myalgia	4 (31)
Nausea	8 (62)
Emesis	5 (38)
WBC shift	12 (92)

1.6 NV Challenge Dose Titration Study and Dose Justification

The challenge strain (GI.1 Norwalk virus strain (8f2), Lot 001-09 NV), was tested at a low and high dose in 8 subjects per cohort in Study VXA-G11-201.1 (n=16). The low dose consisted of a single vial containing $3.6x10^5$ GC and the high dose consisted of 3 vials containing $1.1x10^6$ GC. The viral inoculum was administered with bicarbonate buffer. The 16 volunteers were healthy young adults age 18-49 years old, secretor-positive and blood group A or O. The results of the two challenge cohorts are summarized in **Table 14.**

Five of 8 subjects (62.5%) in both the low and high dose groups met the definition of acute gastroenteritis (AGE). Based on diarrhea and emesis output, the illness was more severe in the high dose group. Symptoms associated with AGE were nausea, abdominal bloating and headache. Low grade fevers ranging from 37.1 to 37.8 °C were observed in the high dose group.

Table 14: Description of illness after challenge with two different doses of Norwalk virus (Study VXA-G11-201.1)

ID Subject Number	Age / Sex	Number Diarrheal Stools	Diarrheal Stool Total wt. (g)	No. Vomiting	Vomiting Total wt. (g)	Max. Temp °C	Time to Onset (hrs)	Duration (hrs)	Clinical Outcome	NV PCR
Low Dose Group ($3.6x10^5$ GC)										
		2	172	2	1125				AGE	POS
		1	138	1	284				AGE	POS
		8	919	0					AGE	POS
		1	53	0					Well	NEG
		2	687	0					AGE	POS
		0		0					Well	NEG
		0		0					Well	NEG
		4	1076	0					AGE	POS
High Dose Group ($1.1x10^6$ GC)										
		1	31	0					Well	POS
		2	547.3	0					AGE	POS
		0		0					Well	NEG
		6	593	1	200				AGE	POS
		5	621.9	0					AGE	NEG
		2	198.8	0					Well	POS
		20	1963	6	1731				AGE	POS
		5	415.4	1	1039				AGE	POS

Clinical Outcome

In the low dose group, 5 (63%) of 8 subjects met the criteria for acute gastroenteritis and were also positive for NV by PCR **(Table 15)**. The other 3 subjects in the cohort were well and NV negative. In the high dose group, 6 (75%) of 8 were NV positive; 4 met the AGE criteria and 2 others had milder diarrhea that did not meet the definition of AGE. One subject was well and NV negative and another subject had diarrhea and was negative for both NV GI.1, GII.4 and other enteric pathogens. Among those subjects who had AGE and were NV positive, the illness was more severe in the high dose group as measured by diarrheal stool output and vomiting. The onset of illness was shorter, and the duration of illness was longer in the high dose group. The Vesikari score was also higher (6.3 vs 4.8) in the high dose group compared to the low dose group and the mean maximum body temperature was also slightly higher in the high dose group **(Table 16)**. The symptoms recorded among the 9 subjects with AGE and who were NV positive were in general mild to moderate in nature (data not shown).

Table 15. Summary of the correlation between acute gastroenteritis and NV positivity by cohort

Category		Dose Cohort		
		Low	High	All
Illness	Infection	n=8	n=8	n=16
AGE	NV+	5 (62.5)	4 (50)	9 (56.3)
Well	NV+	0	2 (25)	2 (12.5
AGE	NV-	0	1 (12.5)	1 (6.3)
Well	NV-	3 (37.5)	1 (12.5)	4 (25)

Table 16. Characterization of diarrhea in subjects with acute gastroenteritis and NV positivity by cohort (by mean)

Characteristic	Low dose	High dose	All subjects
Number of subjects	5	4	9
Number of diarrheal stools	3.4	8.3	5.6
Total weight (g)	599	880	723.7
Number of vomiting episodes	1.5	2.7	2.2
Highest temperature (°C)	37.3	37.5	37.4
Time to onset (hrs)	52.5	36.9	45.6
Duration of diarrhea (hrs)	10	20.7	14.7
Vesikari score	4.8	6.3	5.4

1.7 Summary of Clinical Experience with Vaxart Oral Vaccine Platform

Approximately 640 subjects have been enrolled in 13 clinical trials completed with Vaxart's oral Ad5 vaccine platform. Three phase 1 studies using the Norwalk GI.1 norovirus candidate (VXA-G1.1-NN) have been conducted in 206 subjects. A total of 640 subjects participated including 497 subjects who received Vaxart's oral vaccines at varying dose levels and dosing schedules, across four clinical programs (seasonal and pandemic influenza, norovirus, RSV and coronavirus) and 143 subjects received placebo. To date, the vaccines were well tolerated, and in the placebo-controlled studies, the vaccine groups, at all dose levels, did not differ significantly in the frequency of adverse events (aEs) recorded from the placebo groups. A summary of the clinical studies conducted using Vaxart's vaccine platform is presented in **Table 17**.

Table 17: Clinical Studies Completed Using Vaxart Oral Ad5 Vaccine Platform

Target Pathogen	Vaccine Candidate(s)	Protocol Number and (Oral Vaccine Formulation)	Year Study Completed (Active Phase)	Subjects with ≥ 1 Vaccine Exposure	Subjects Receiving Placebo
Influenza H5	**ND1.1**	VXA01-001 (H5 Capsule)	2011	42	12
		VXA01-001A (H5 RCC)	2012	12	-
Influenza H1	**VXA-A1.1**	VXA02-001 (H1 Tablet)	2012	25	12
		VXA02-002 (H1 RCC)	2013	37	-
		VXA02-003 (H1 Tablet)	2014	12	12
		VXA-CHAL-201 (H1 Tablet) [BARDA Funded]	2017	71	36
		VXA-RLT-1 (H1 Fast/Fed)	2018	8	-
Influenza B	**VXA-BYW.10**	VXA03-001 (Flu B Tablet)	2016	38	16
Norovirus GI.1	**VXA-G1.1-NN**	VXA-G11-101 (NV Tablet)	2016	46	20
		VXA-G11-102 (NV Tablet)	2017	60	-
Norovirus GI.1 and GII.4	**VXA-G1.1-NN** and **VXA-G2.4-NS**	VXA-NVV-103 (NV Tablet)	2019	65	15
RSV	**VXA-RSV-f**	VXA-RSV-101 (RSV Tablet)	2016	46	20
Coronavirus	**VXA-CoV2-1**	VXA-COV2-101 (SARS-CoV-2 S & N Tablet)	2021	35	0
Total Subjects Dosed				**497**	**143**

Ad5 = serotype 5 adenovirus; BARDA = Biomedical Advanced Research and Development Authority; BB-IND = biologic-based investigational new drug; NV = norovirus; RCC = Radio-Controlled Capsule; RSV = respiratory syncytial virus

Safety in Vaxart's oral vaccine programs has been assessed by examining the incidence of solicited symptoms of reactogenicity, aEs and serious aEs (SAEs) in the intent-to-treat (ITT) population of each study. Solicited local and systemic aEs were collected for 7 days following each vaccine administration using subject diary cards. Unsolicited aEs were collected for 28 days following last vaccination. Additionally, SAEs, aEs of special interest (AESIs) and new onset of chronic illness (NOCIs) were monitored throughout the one-year safety follow-up period. No related SAEs, AESIs or NOCIs have been reported for any subject receiving single or multiple doses of Vaxart's VAAST™ oral vaccines to date.

1.8 Rationale for GI.1 Human Challenge Study (VXA-NVV-201)

The human challenge model has been used successfully to determine the efficacy of both GI.11 and GII.4 norovirus vaccines (30, 31). The challenge with GI.1 was more successful because the GI.1 challenge induced illness in more control subjects. The challenge model has been called into question as an overly stringent model because the inoculum used in these studies induces illness in over half of the unvaccinated control subjects. In nature the dose may be much lower. For example, in the Atmar study using the GI.1 challenge the protective efficacy of a parenteral GI.1 NV vaccine was 47%. The hope is that the efficacy will be considerably higher in field studies. The challenge study may also provide immunological data that may aid in determining an immunologic correlate of protection. A correlate of protection could greatly aid in predicting vaccine efficacy in different age groups. An effective Norovirus vaccine must prevent the two most common norovirus genotypes, GI.1 and GII.4. Vaxart is developing a bivalent vaccine for use in the field.

1.9 Potential Risks and Benefits

1.9.1 Vaccine Risks

Vaxart's Ad5 vector is a replication incompetent vaccine, lacking the E1 and E3 gene regions, which have been removed recombinantly. Replication incompetent Ad5 has been used in over 200 gene therapy and vaccine studies in humans (36-39). VXA-G1.1-NN is an investigational new drug. Similar doses of the related investigational drugs (vaccines) ND1.1 and VXA-A1.1 have been tested in prior phase 1 and phase 2 studies; the numbers were relatively small (n = ~500 subjects). Based on the results from 3 studies completed with the VXA G1.1-NN norovirus vaccine, doses of 2 x 10^{11} ±0.5 logs, delivered by oral enteric tablet were safe and generally well tolerated in healthy male and female subjects.

The safety monitoring practices employed by this protocol (ie, physical examination, vital signsgastrointestinal and systemic reactogenicity (solicited symptoms), 12-lead electrocardiogram (ECG), hematology, serum chemistry, urinalysis, immunogenicity assessments, and AE questioning) are adequate to protect the subjects' safety and should detect all expected treatment-emergent AEs (TEAEs).

Since the initiation of the current protocol, multiple injected adenovirus vectored vaccines approved for use via Emergency Use Authorization (EUA) for the prevention of COVID-19 have reported severe events of thrombosis in combination with thrombocytopenia (thrombosis with thrombocytopenia syndrome, TTS), in some cases accompanied by bleeding. Though the TTS events were reported with adenovirus vectored vaccines for COVID-19, the FDA has asked Sponsors of adenovirus vectored vaccines for other indications to broadly monitor for these risks.

Investigators should be alert to the signs and symptoms of thromboembolism and/or thrombocytopenia. Study participants should be instructed to seek immediate medical attention if they develop symptoms

including, but not limited to, shortness of breath, chest pain, leg pain and/or swelling, persistent abdominal pain, severe or persistent headaches, blurred vision or other vision changes, mental status changes or seizures, petechia, purpura beyond the site of vaccination, and/or easy bruising/bleeding. The medical management of thrombosis with thrombocytopenia is different from the management of isolated thromboembolic diseases. Investigators should follow available guidelines for the assessment and treatment of thrombotic thrombocytopenia (e.g., American Society of Hematology 2021British Society for Haematology 2021; CDC 2021). The use of heparin may be harmful and alternative treatments may be needed. Consultation with hematologists is strongly recommended.

1.9.2 Norovirus Challenge Risks

The intended challenge NV dose of $1x10^6$ GC is expected to elicit acute watery diarrhea within 12-72 hours of ingestion in about half of the subjects. Persons that are blood group A or O and secretor-positive are known to be more susceptible to NV. Nausea or vomiting, abdominal cramping or discomfort, and loss of appetite are also common symptoms. Fever is uncommon. Dehydration can occur through loss of fluid from liquid stool and emesis. Since NoV infection is short-lived lasting usually 2 or 3 days severe dehydration is uncommon. Symptoms of severe dehydration can involve dry mouth, decreased urine, thirst, cold clammy skin, hypotension, lethargy, stupor, and muscle cramping. The complications of severe fluid loss include hypoglycemia (low blood sugar), acidosis, kidney failure, pulmonary edema (fluid in lungs), arrhythmia (heart rhythm abnormalities), coma, and death. Dehydration will be managed with aggressive fluid rehydration (oral and/or intravenous [IV]) with potassium repletion. It is expected that NoV shedding will occur in the stool; however, transmission from person to person is mitigated by housing the subjects on the research isolation ward until they meet the protocol discharge criteria. Subjects that maintain the standard hygiene (ie, hand washing with soap and water after defecation) are unlikely to transmit the strain person to person.

1.9.3 Risk of Blood Draw

The approximate volume of blood planned for collection from each subject over the course of the study presents no undue risk to the subjects nor does the possibility of collection (for wasting to ensure clean sample) of additional blood in the event an indwelling cannula is utilized and the possibility of additional blood for recheck of safety labs if deemed necessary by the Principal Investigator (PI).

1.9.4 Known Potential Benefits

Since these are experimental vaccines against norovirus, there are no benefits to the subjects for their participation in this research study.

2 HYPOTHESIS, OBJECTIVES AND PURPOSE

2.1 Study Hypothesis

Norovirus vaccine (VXA-G1.1-NN) will protect against Norovirus Gastroenteritis (NVG) in the challenge model.

2.2 Study Objectives

2.2.1 Primary Objective

- Determine the clinical efficacy of VXA-G1.1-NN compared to placebo, to protect against NVG caused by the Norwalk strain challenge inoculum.

2.2.2 Secondary Objectives

- Safety and tolerability of VXA-G1.1-NN oral vaccine

- Determine the ability of VXA-G1.1-NN to modify disease severity (defined in **Appendices C and D**) compared to placebo
- Determine the quantity and duration of norovirus shedding by qRT PCR
- Evaluate the VP1 specific IgA ASC, HBGA blocking antibody, and VP1 specific serum IgG responses to VXA-G1.1-NN

2.2.3 Exploratory Objectives

- Determine correlation of immunogenicity parameters with clinical outcome
- Further evaluate immunogenicity of VXA-G1.1-NN

3 STUDY DESIGN AND ENDPOINTS

3.1 Description of Study Design

This is a Phase 2b randomized, double-blind, placebo-controlled vaccination and challenge study to assess the protective efficacy of the Norovirus vaccine (VXA-G1.1-NN). To ensure at least 140 subjects (approximately 85 NV vaccine and 85 placebo) are available to participate in the challenge phase, approximately 170 healthy young adults will be randomized in a 1:1 ratio to receive one oral dose of vaccine or placebo **Table 18.** Each subject will receive an oral dose of $1x10^{11}$ IU±0.5 log VXA-GI.1-NN or placebo comprised of multiple tablets per dose.

To accommodate the limited size of the isolation unit that will be utilized for the challenge and post-challenge isolation period, subjects will move through the study (enrollment, vaccination and challenge) sequentially in a total of approximately 14 to 16 cohorts. Subjects will be randomized in cohorts of approximately 10 to 12 each. Approximately 28 days (+30 days) post-vaccination, each cohort will be admitted to the isolation ward and challenged with the NV GI.1 Norwalk challenge strain. After challenge, subjects will be monitored for signs and symptoms of AGE from Day 29 to discharge. Stool and emesis will be collected. Excretion of NV (shedding) will be monitored in stool and emesis. Illness will be assessed using a modified Vesikari scale. NV illness lasts 2-4 days and is self-limited.

Four days post-challenge (Day 33) subjects will be evaluated for signs and symptoms of acute gastroenteritis. If there is no clinical illness present, asymptomatic subjects will be discharged from the isolation ward and will be followed up in a series of outpatient visits and telephone calls. Symptomatic subjects will remain in the unit up to an additional 3 days (Day 36), per the judgement of the investigator.
An independent Safety Monitoring Committee (SMC) will convene at pre-defined intervals during the norovirus challenge period, and also ad hoc as needed during the vaccination and challenge periods, to oversee the safety of the study.

Each subject's participation will last approximately 1 year post successful screening and enrollment

- 90 day pre-screening period
- 45 day screening period
- 28 day vaccination phase
- 28 day viral challenge and follow-up
- 365 day total safety follow-up post vaccination

Table 18: Study Design and Vaccine Groups (Study VXA-NVV-201)

Product/Test Agent	No. of subjects
VXA-G1.1-NN oral vaccine tablets [$1x10^{11}$ IU±0.5 log]	85
Placebo identical to NVV	85
Challenge Norwalk Virus Strain [Lot 001-09NV and Sublot 2 ($1x10^6$ GC)]	140*

**All subjects suitable to undergo challenge at Day 29 will participate in the challenge phase.*

3.2 Study Endpoints

3.2.1 Primary Endpoint

- Occurrence of NVG caused by the challenge norovirus during inpatient challenge period.
  - NVG is a composite endpoint and is defined as meeting one or more of the definitions for both acute gastroenteritis and NV infection.

3.2.2 Secondary Endpoints

- Number of subjects with acute gastroenteritis during the inpatient challenge period
- Mean score using the Modified Vesikari Scale as a measure of severity of acute gastroenteritis post-challenge at discharge (up to Day 36)
- Number of subjects with moderate or severe gastroenteritis (cumulative loose stools ≥ 1000gms during the inpatient period)
- Median duration (number of hours) of acute gastroenteritis among challenged subjects
- Number of subjects with norovirus infection up to Day 36 post-challenge (quantitative reverse transcriptase polymerase chain reaction [qRT-PCR] positive in stool, Norwalk virus antigen in stool
- Geometric mean genome copies of norovirus shedding by qRT-PCR
- Median duration (number of hours) of norovirus infection among challenge subjects
- Number of incidences of diarrhea or emesis among challenge subjects

3.2.3 Safety Endpoints

- Vaccine (VXA-G11-N.N)
  - Solicited symptoms of reactogenicity x 7 days post vaccination;
  - Unsolicited AEs x 28 days post vaccination;
  - SAEs, AESIs and NOCIs x 12 months post vaccination

- Challenge Virus (Norwalk GI.1)
  - Unsolicited AEs x 28 days post challenge

3.2.4 Immunogenicity Endpoints

Primary

- VP1 specific IgA ASC response against Norwalk at Day 8 compared to placebo
- GMT of HBGA blocking antibodies (by BT_{50}) against Norwalk virus at Day 28 compared to placebo
- VP1 specific serum IgG response at Day 28 compared to placebo
- VP1 specific serum IgA at Day 28 compared to placebo

Exploratory

- Correlations between individual immunogenicity parameters and clinical efficacy

- VP1 specific IgG ASC response against Norwalk at Day 8 compared to placebo
- VP1 specific fecal IgA at Day 28 compared to placebo
- VP1 specific saliva IgA at Day 8 and Day 28 compared to placebo
- VP1 specific nasal IgA at Day 8 and Day 28 compared to placebo
- Vaccine specific response for memory IgA and/or IgG in B cells, IgA and IgG in plasmablasts, antibody repertoire sequencing analysis, flow cytometry (including homing and/or immunophenotyping analyses), and other assays designed to evaluate cell mediated immunity

Additional assessment of serum, fecal, saliva, and mucosal immunity at other time points (including post-challenge) may be performed

4 STUDY ELIGIBILITY, ENROLLMENT AND WITHDRAWAL

4.1 Subject Inclusion Criteria

1. Male or female between the ages of 18 – 49 years, inclusive
2. Able to give written informed consent
3. Healthy[1], as determined by the principal investigator (PI) or PI in consultation with the Medical Monitor and Sponsor

   [1]*No clinically significant health concerns or medical illness), as determined by medical history, physical examination, vital signs, electrocardiogram (ECG), and clinical laboratories (complete blood count [CBC], chemistry and urinalysis)*
4. Comprehension of the study requirements with ability and willingness to complete all assessments and comply with confinement period post viral challenge, and all scheduled visits and contacts
5. Confirmed blood type (A or O)
6. Demonstrated to be H type-1 antigen secretor positive (by saliva test)
7. Body mass index between 17 and 35 kg/m^2, inclusive, at Screening
8. Female participants must have a negative pregnancy test at pre-vaccination and pre-challenge and fulfill one of the following[2]

   [2]*Criteria:*
   - *a. At least one year post-menopausal;*
   - *b. Surgically sterile;*
   - *c. Use of oral, implantable, transdermal, intravaginal or injectable contraceptives for 30 days prior to immunization and until 60 days after challenge;*
     - *i. A reliable form of contraception must be approved by the Investigator (eg, double barrier method, Depo-Provera, intrauterine device, Norplant, oral contraceptives, contraceptive patches, vaginal ring contraceptive)*
   - *d. Not be sexually active (abstinent) or in a same sex relationship (must be discussed with site staff and documented)*
9. Male subjects must agree not to father a child or donate sperm, as well as to use contraception/barrier (a male condom) or be abstinent from heterosexual intercourse, from vaccination through the active/interventional period of the study (Day 57)

4.2 Subject Exclusion Criteria

1. Administration/use of any investigational drug or device 30 days prior to vaccination through the active period (Day 57)
2. Administration of any licensed vaccine within 30 days prior to vaccination or planned use of the above stated during the active period (through Day 57)
3. Presence of a significant medical condition[3] which in the opinion of the investigator precludes study participation[3]

   [3] *For example, psychiatric conditions, or gastrointestinal disease, such as peptic ulcer, symptoms or evidence of active gastritis or gastroesophageal reflux disease, inflammatory bowel disease, alcohol or illicit drug abuse/dependency, or other laboratory abnormalities*

4. Laboratory values outside the range of normal for platelet counts and the following coagulation tests: PT/INR, aPTT and fibrinogen
5. Any of the following history or conditions that may lead to higher risk of clotting events and/or thrombocytopenia:
   e. Family or personal history of bleeding or thrombosis
   f. History of heparin-related thrombotic events, and/or receiving heparin treatments
   g. History of autoimmune or inflammatory disease
   h. Presence of any of the following conditions known to increase risk of thrombosis within 6 months prior to screening:
      - Recent surgery other than removal/biopsy of cutaneous lesions
      - Immobility (confined to bed or wheelchair for 3 or more successive days)
      - Head trauma with loss of consciousness or documented brain injury
      - Receipt of anticoagulants for prophylaxis of thrombosis
      - Recent clinically significant infection
6. Any one of the following ECG findings[4] within 45 days prior to vaccination:
   [4] *Exclusionary ECG findings:*
   a. QTc F (interval duration > 450 msec (male) or > 470 msec (female)
   b. QRS interval greater than 120 msec
   c. PR interval greater than 220 msec
   d. Clinically significant ST-T wave changes or pathologic Q waves
7. History of cancer or cancer treatment within past 3 years (excluding basal cell or squamous cell carcinomas)
8. Presence of immunosuppression or medical condition possibly associated with impaired immune responsiveness, including diabetes mellitus or angioedema
9. Donation or use of blood or blood products within 30 days prior to vaccination through the active period (Day 57)
10. Diagnosed bleeding disorder or significant bruising or bleeding difficulties that could make blood draws problematic
11. Any condition that resulted in the absence or removal of the spleen

12. Evidence of confirmed infection with human immunodeficiency virus (HIV), hepatitis B surface antigen (HBsAg) or hepatitis C virus (HCV) with confirmatory assays
13. Abnormal stool pattern (fewer than 3 bowel movements per week or more than 3 per day)
14. History of irritable bowel disease or inflammatory digestive or gastrointestinal condition[5] that could affect the distribution / safety evaluation of an orally administered vaccine[6]

    [5]*Such conditions may include but are not limited to:*

    a. *Esophageal Motility Disorder*
    b. *Malignancy*
    c. *Malabsorption(eg. Celiac disease, gluten intolerance)*
    d. *Pancreaticobiliary disorders*
    e. *Irritable bowel syndrome*
    f. *Inflammatory Bowel Disease*
    g. *Surgical Resection with the exception of appendectomy or a minor resection that is deemed acceptable by investigator and sponsor*
    h. *Gastroesophageal reflux disease (GERD)*
    i. *Hiatal Hernia*
    j. *Peptic Ulcer*

    (History of cholecystectomy is not exclusionary)

    [6] *targeting the mucosa of the small intestine*

15. Use of proton pump inhibitors, H2 blockers or antacids within 7 days prior to vaccination through the active period (Day 57)
16. Use of antibiotics within 30 days prior to vaccination through the active period (Day 57)[7]

    [7]Note: use of a brief *(≤ 10 days) course of oral or topical antibiotic for minor URI, UTI, dental work, or skin infection allowed within the screening period, but must be completed 7 days prior to first vaccination*
17. Use of medication known to affect the immune function (e.g. systemic corticosteroids and others) within 14 days prior to vaccination through the active period (Day 57)
18. Regular use of nonsteroidal anti-inflammatory drugs within 7 days prior to vaccination through the active period (Day 57)
19. Use of over-the-counter probiotics or antidiarrheals within 7 days prior to vaccination through the active period (Day 57)
20. Evidence of recent (within 2 months of vaccination) or of current nonbacterial gastroenteritis suggestive of NV infection [vomiting or unformed or watery stools (> 2 during a 24-hour period)]
21. Any gastroenteritis within the past 2 weeks prior to vaccination
22. Acute disease within 72 hours prior to vaccination[8]

    [8]*Defined as the presence of a moderate or severe illness with or without fever (as determined by the Investigator through medical history and physical examination). (Assessment may be repeated during screening period)*
23. Presence of a fever ≥ 38ºC measured orally at baseline
24. History if hematochezia (blood in stool) or melena (black stool)
25. Any significant hospitalization within the last year which in the opinion of the investigator or sponsor could interfere with study participation

26. History of serious reactions to any vaccination such as anaphylaxis, respiratory problems, Guillain-Barre syndrome, hives or abdominal pain
27. History of a hypersensitivity or allergic reaction to any component of the investigational vaccine or placebo[9]. Subjects with known fish allergies should be excluded

    [9] *including but not limited to fish gelatin*
28. History of drug, alcohol or chemical abuse within 1 year prior to vaccination
29. Positive test for drugs of abuse or alcohol at Screening, vaccination baseline and pre-challenge.[10]

    [10]*except for previous marijuana use; concurrent or ongoing use of marijuana during the active study period*
30. Consistent/habitual smoking within 2 months prior to vaccination (defined as smoking ≥ 1 pack of cigarettes a day). Smoking is not permitted during the inpatient stay
31. Other conditions[11] that would jeopardize the safety or rights of a subject or interfere with the evaluation of the study

    [11] *in the clinical judgment of the investigator*

Social/Occupational:

32. Living with or having daily contact with children age ≤ 5 years old or women known to be pregnant or nursing[12]

[12]*This includes significant contact at home, school, day-care, or equivalent facilities.*

33. Living with or having daily contact with elderly persons ≥ 70 years of age or infirmed, diapered individuals, persons with disabilities or incontinence[13]

[13]*This includes at work or visits to nursing homes and day-care or equivalent facilities*

34. Employment in the food service industry such as restaurant or cafeteria facilities[14]

    [14]*Specifically, this includes persons whose employment requires food handling and processing in the 4 weeks following viral challenge*
35. Health-care workers with patient contact expected in the 4 weeks following viral challenge
36. Expected contact[15] with immunocompromised persons[16] in the 4 weeks following viral challenge

    [15] *via employment or at home*

    [16] *HIV-positive, receiving immunosuppressive medications such as oral steroids, anti-neoplastic agents*
37. Presence of household members who have received the Ad4 or Ad7 vaccines within 2 months prior to vaccination
38. Employment as an airline flight attendant or cruise ship crew, scheduled to work in the 4 weeks following challenge.
39. Persons planning to live in a confined environment (eg, a cruise, camp, etc.) in the 4 weeks following viral challenge

4.3 Withdrawal from the Study

4.3.1 Reasons for Withdrawal or Termination

Participation in the study is strictly voluntary. Subjects have the right to withdraw from the study at any time and for any reason, without penalty. The Principal Investigator and/or designee may, at her/his discretion, withdraw a subject from continuing in the study if it is in the subject's best interest, or if the

subject is not willing or able to comply with the study requirements. The reason for withdrawal will be documented.

4.3.2 Handling of Subject Withdrawals or Termination

If a subject withdraws or early terminates on study, every effort will be made to complete protocol-specified safety follow-up procedures, with the consent of the subject, to capture protocol specified assessments and AEs, SAEs, AESIs and NOCIs (refer to Section 7.11).

5 STUDY DRUG

5.1 Study Drug(s) and Control Description

VXA-G1.1-NN final DP is produced as small white enteric-coated tablets **Table 19**. The DP is formulated as compressed powdered solid material containing lyophilized drug substance (VXA-G1.1-NN), tromethamine (TRIS), sucrose, arginine, hydrolyzed fish gelatin (type A), polyvinylpyrrolidone (PVP-grade K90), microcrystalline cellulose, pre-gelatinized starch, magnesium stearate, hydrophobic colloidal silica, and hydrochloric acid for pH adjustment. With the exception of the active ingredient all excipients are used in US-licensed oral DPs currently listed in the FDA Inactive Ingredients Database and are GRAS categorized.

Table 19: Active Product Description (VXA-G1.1-NN)

Generic name	VXA-G1.1-NN
Chemical name	Ad-CMV-VP1(Norwalk virus)-BGH-CMV-luc dsRNA-SPA
Genome size	33,949 bp dsRNA genome
Viral particle size	80 -100 nm

Placebo Description

The placebo for this study is manufactured similarly to the active DP tablets, but without the active drug substance. The placebo tablets are indistinguishable in appearance from the active DP tablets. The number of placebo tablets dispensed to the subject will be matched to the active treatment groups. The placebo will be dispensed by the site's in-house pharmacy in a manner indistinguishable from the active treatment groups.

5.1.1 Drug Product Packaging and Labeling

DP tablets will be packaged into foil-sealed, HDPE screw-cap containers with 10 tablets per bottle. These will be provided to the clinical site pharmacy in an unblinded manner.

All lots of DP bottles will be labeled with the name of the manufacturer, the protocol number, the name of the product, the lot number of the product, the concentration of the vaccine, the date of manufacturing and any additional information as required by regulatory agencies or clinical pharmacy Standard Operating Procedures (SOP).

Secondary packaging of the DP upon dispensing from the pharmacy to the clinical staff for subject dosing will be determined with consideration of the sites' pharmacy SOPs and outlined in the study pharmacy manual. The final subject use material (cup or secondary bottle) will be appropriately labeled with the subject's unique identifier, the time/date of dose preparation within the pharmacy, an expiration time and date, and additional information as deemed necessary.

5.1.2 Drug Product Storage and Stability

VXA-G1.1-NN vaccine and placebo tablets will be stored at 2 to 8°C at the clinical site until ready for use. Test data on representative DP lots support the stability of the DP tablets stored at 2 to 8°C for at least 1 year from date of manufacture prior to retest. Additionally, data from accelerated stability studies indicate that handling at controlled room temperature or brief exposure to temperatures above room temperature (below 37 °C) is acceptable for this product.

DP tablets should not be frozen. Do not use and contact Sponsor if DP tablets have been frozen.

5.1.3 Drug Product Dosage Preparation and Administration

Preparation of Drug Product Dose

DP doses will be prepared at the site by the unblinded pharmacy staff for delivery to the clinical staff in a blinded manner. The observed concentration of VXA-G1.1-NN active ingredient per tablet has been within the range of $2x10^9$ IU – $5x10^{10}$ IU. The actual concentration of active vaccine per tablet is determined during release testing and provided to clinical sites within the Certificate of Analyses (CoAs). Multiple tablets of the DP will be dispensed per dose to deliver the complete investigational dose of $1x10^{11}$ IU ± 0.5 logs within the vaccine treatment arm. A matching number of placebo tablets will be administered to ensure blinding of the treatment groups. Refer to the Study Pharmacy Manual for detailed information on the drug product lots to be used for clinical dosing and the number of tablets to be administered.

At least 15 minutes prior to administration, the dose container(s) will be removed from the refrigerator and allowed to equilibrate with room temperature. Multiple subject doses may be withdrawn from the container with proper drug accountability. If tablets remain in the primary bottle container upon preparation of subject doses, the desiccant pack should be replaced, and the container tightly capped before being returned to the refrigerator. The DP containers may be held at ambient room temperature for no more than 4 hours prior to returning it to the refrigerator. The containers should be placed in a dry location that is free from extreme sources of heat or light.

Study Drug Administration

Study subjects should be fasting and refrain from ingesting solid food for at least 4 hours prior to initiation of oral dosing. Following randomization subjects will be dispensed their assigned DP dose (VXA-G1.1-NN or placebo tablets) on Day 1. They will swallow the tablets with 360 to 480 mL of water followed by a light snack (e.g., crackers) to aid in tablet transit out of the stomach. Normal food consumption may resume 90 minutes after dosing.

Dose Adjustments/Modifications/Delays

Dose adjustments and or modifications are not planned or allowed under this clinical protocol. All subjects should receive the full $1x10^{11}$ IU ± 0.5 logs dose or matching placebo at Day 1. Any modification from this planned dose should be recorded as a protocol deviation and reported to the Sponsor (or designee).

5.1.4 Study Drug Accountability

The Investigator and study site must ensure that an accurate record of DP disposition is maintained, and that study drug is dispensed only by authorized, unblinded personnel as documented on the delegation of authority log. Records of product disposition and dispensing must consist at a minimum of the date received, date administered, quantity administered, and the subject to whom the drug was administered.

The unblinded pharmacy staff will be responsible for maintaining accurate records of the shipment and dispensing of the study drug. The investigational product records must be available for inspection by the Sponsor's unblinded representative and is subject to inspection by a regulatory agency at any time. Copies of the records will be provided to the Sponsor at the end of the study. An unblinded study monitor will review the investigational product records.

Clinical Trials Materials Returns form and the drug accountability forms must be maintained in a blinded secure area throughout the duration of the study. At the completion of the study, all unused product must be returned to the Sponsor or designee, per instruction by the Sponsor.

6 NV CHALLENGE INOCULUM

The Norovirus GI.1 (Norwalk Virus Inoculum Lot 001-09NV, IND 14697) inoculum was prepared from a 20% suspension of a liquid stool from a NV-infected subject in a previous human challenge study. This inoculum was prepared by extracting the virus from the stool suspension after several washes, sterilely filtering the virus extract into vials, and determining the titer of the extract. These procedures were performed under Good Manufacturing Practices conditions at [redacted] From there, the inoculum was sent to an outside contractor for safety testing. The preparation, qualification, and quantification of this inoculum were submitted to the FDA under IND 14697.

To accommodate the increased sample size of the trial, additional GI.1 norovirus inoculum was diluted from the same original master stock Norwalk Virus Inoculum Lot 001-09NV purified bulk harvest (PBH), to challenge the additional Subjects. The dilution process is attached as Appendix F and an updated IND 14697 subsequently submitted to the FDA with sterility, endotoxin, and titer testing results.

6.1 Formulation, Packaging, and Labeling of NV Challenge Virus

Both the filled, purified product and the final patient use material are formulated in pure, distilled water with no other additives. The filled, purified material is provided to the clinical site in 1 mL sterile cryovials containing sufficient material for one final processing step just prior to use in a single subject. The filled, purified NV inoculum is labeled with the name of the manufacturer, the name of the product, the lot number of the product, the concentration of the virus, the date of manufacturing and a statement stating For Investigational Use Only.

The Sublot 2 inoculum material diluted and prepared for single use to complete this challenge study was packaged in one 3mL cryovials. The dilution and aliquoting of virus were performed at [redacted] with oversight by a representative from the original manufacturing team following the dilution procedure described in the Preparation of NV Challenge Inoculum Work Instruction, Appendix F. During the aliquoting of 001-09NV sublot 2, additional vials from the beginning, middle and end of the run were prepared for quality control testing and shipped on dry ice to either [redacted] or [redacted] for testing.

6.2 Storage and Stability of NV Challenge Virus

The filled, purified material is stored long term in a controlled -80°C freezer. The final subject use material can be stored short term in either a 4°C refrigerator or can be held short term on wet ice. The final subject material is not held or stored for more than 8 hours. The long-term stability of frozen clinical-grade NoV prepared using the established procedures at the NoV Processing Facility in North Carolina has not been determined. Stability will be determined by periodically removing a vial of the filled, purified material from produced banks of vials and testing the material for sterility and viral titer via RNA qRT-PCR. The stability of the final subject use material has been pre-set at 8 hours.

The final titer for 001-09NV PBH was 2.4 x10e6 genome copies (GC)/mL. The titer in 2009 was 5 x 10e6 GC/mL. Titer of 001-09NV Final Fill sublot 1, a 1/10 dilution of 001-09NV PBH was 5 x 10e5 GC/mL in 2009 and 2.9 x 10e5 in 2022. These results indicate the virus stock has been stable for more than 10 years.

Additional vials of 001-09NV Final Fill sublot 2 were tested to better evaluate the equal distribution of the virus across the dilution aliquot run. The mean of all dilutions on the standard curve that passed

QC, for all nine vials across three independent assays was 5.36 10e5 GC/ml. The titer was consistent across the aliquoting run. Notably, the titer of 001-09NV sublot 1 ranged from 6 x 10e5 GC/mL to 3 x 10e5 GC/mL between 2009 and 2022, indicating that 001-09NV Final Fill sublot 2 is performing consistently with 001-09NV Final Fill sublot 1. The reported titer is slightly higher than expected from the 1/8 dilution of master stock but falls within the established range of titer based on historic results of 001-09NV Final Fill sublot 1 (5 x 10e5 GC/mL +/- 2 x 10e5 GC/mL).

6.3 Preparation and Administration of NV Challenge Virus

Subjects will receive a single oral dose of safety-tested inoculum approximately 28 days (+30 day window) post vaccination. The oral route was chosen because of ease of administration and successful precedent with this route from all other NoV challenge studies.

Subjects will be challenged with a single oral dose of $1.1x10^6$ GC of NV based on information from the NV GI.1 dose titration study VXA-G11-201.1, past challenge studies and the NV dose-response model (see **Section 1.6**). The dose-response model and experience with this dose indicates that this dose is sufficient to result in an infection rate between 50% to 65% among susceptible, secretor-positive status individuals. The dose is a single-subject dose (three 1-mL vials for a total of 3 mL of NV inoculum Lot 001-09NV, or one 3 mL vial for Sublot 2, IND 14697)

Three (3) or one (1) vial(s) per subject will be thawed under sterile conditions and swabbed with disinfectant. The inoculum vial(s) will be added to 100 mL of distilled water in a sterile, disposable, sealable cup under sterile conditions. After mixing, the solution will look like a plastic glass of clear drinking water. The sealed cup will be transported on ice to the subject for challenge.

Since the inoculum contains live infectious human pathogen, biohazard precautions will be observed. This includes disposing of all waste in biohazard bags, cleaning equipment and materials that have come into potential contact with the inocula with 10% bleach solution. Cups used for administration and any leftover inocula should also be cleaned with bleach and discarded. If the subject, person administering dose, or person preparing inoculum spills the inoculum, the affected area must be immediately cleaned with a 10% bleach solution. Hands should be washed thoroughly with soap and water.

Partially used vials may not be used for human administration or for in vitro experimental studies. At the end of the study, the site will receive instruction from the Sponsor regarding the final disposition of any remaining NV inoculum.

7 STUDY PROCEDURES AND SCHEDULE

Refer to the APPENDIX A: SCHEDULE OF EVENTS

7.1 Pre-Screening Period (Day -90 to Screening)

Subjects will be pre-screened for purposes of ascertaining H type-1 antigen secretory status and blood type. Subjects who are eligible for screening based on the results of the pre-screening assessments or known secretory status and blood type will be contacted by telephone and invited to return to the site to sign the ICF and enter the study specific screening period.

7.2 Visit 00: Screening Visit (Study Day -45 to -1)

If the subject is secretor positive (genetically susceptible) and blood type A or O, he or she will be invited to a full screening visit at the outpatient visit site.

At this screening visit, the Study Investigator will perform a clinical assessment and blood will be collected for laboratory tests. At the time of screening, blood cell counts, blood chemistry, liver function, HIV, HBsAg, and HCV status will be tested: a pregnancy test will also be conducted for female participants. Vital signs will also be collected (temperature, blood pressure, heart rate, breathing rate) as well as height and weight for BMI calculation. A medical history and review of concomitant medications will also be performed.

The Study Investigator will provide counseling as needed to subjects on the results of these tests, especially on HIV, HBV, and HCV status. Medical history, physical exam, vital signs and lab results must be within normal limits, or abnormalities deemed not clinically significant, at the time of screening for the subjects to participate in the study. Subjects who are eligible to participate in the study will be contacted for enrollment.

The following procedures will be performed to determine eligibility:

- Informed consent
- Demographics
- Medical history
- Eligibility criteria
- Physical examination
- Review of concomitant medications
- Vital signs (including height and weight)
- ECG
- Blood screen (HIV, HBsAg, HCV)
- Safety labs
    - CBC: Hemoglobin, Hematocrit, Platelet Count and Complete White Blood Cell Count
    - Serum Chemistry: ALT, AST, Total Bilirubin, BUN, Creatinine, Random Glucose, Potassium and Sodium
    - Urinalysis: Glucose, Protein and Hemoglobin
- Blood Sample: coagulation tests, lab panel (PT/INR, aPTT and fibrinogen)
- Female pregnancy test (serum)
- Drug screen (urine and alcohol breath test)

Subjects will be reminded to fast and refrain from ingesting solid food for at least 4 hours prior to the Day 1 study drug administration.

7.3 Visit 01: Study Day 1, Study Drug Administration

Subjects will undergo the following assessments and procedures prior to randomization and study drug administration:

- Assess inclusion/exclusion criteria
- Physical examination (targeted)
- Vital signs
- Safety Labs
    - CBC
    - Serum Chemistry

- Urinalysis

(Note: Safety labs do not need to be performed if screening safety lab tests were completed within 2 days prior to study drug administration on Day 1)

- Female pregnancy test (urine)
- Drug screen (urine and alcohol breath test)
- Blood samples for immunogenicity (PBMCs, serum and fixed whole blood)
- Fecal sample for immunogenicity
- Saliva sample for immunogenicity
- SAM (Synthetic Absorptive Matrix) for nasal mucosal lining fluid (MLF) evaluation
- Review of concomitant medications
- Medical history

Refer to the Laboratory Manuals for details regarding sample collection and handling.

Study eligibility will be reviewed and re-confirmed at this study visit. Those subjects who satisfy all inclusion criteria and none of the exclusion criteria will qualify and be eligible to be randomized into the study.

The following procedures will be performed following randomization:

- Study drug administration (vaccine or placebo) – administer study drug tablets, after subject has been fasting for at least 4 hours prior to start of dosing
  (please refer to Pharmacy Manual for detailed study drug administration instructions)
- Solicited symptoms of reactogenicity e-Diary (electronic Diary) – dispense e-Diary to subject and provide instructions for use
- Assess for solicited and unsolicited adverse events after initiation of study drug administration

7.4 Visit 02: Study Day 8

Every effort should be undertaken to ensure the subject returns to the site on Day 8, exactly 7 days post vaccination, to allow optimal evaluation of immune response. Subjects will undergo the following assessments and procedures:

- Physical examination (targeted)
- Vital signs
- Safety Labs
  - CBC
  - Serum Chemistry
  - Urinalysis
- Review and collect solicited symptoms of reactogenicity e-Diary
- Review of concomitant medications
- Query for AEs
- Blood samples for immunogenicity (PBMC and fixed whole blood)
- Saliva sample for immunogenicity
- SAM (nasal MLF evaluation)

7.5 Visit 03: Study Day 28, 1 day prior to challenge [+3 days for visit window and +30 days window for repeat Visit 03]

All subjects will return to the site at Day 28 for safety assessments and collection of samples to complete the vaccination phase of the study.

Study subjects will begin their inpatient stay 1 day before challenge. Subjects will be assigned to a single room and instructed about the protocol-required procedures (e.g., stool handling), hygiene practices, and the "Rules and Procedures" to be followed while in the inpatient unit. Subjects will also be monitored for evidence of behaviors which might pose a safety risk to themselves, other subjects, or staff and could be cause for ineligibility for challenge and the remainder of the inpatient stay. Refusal to comply with protocol-required procedures, adherence to hygiene practices could constitute ineligibility

Any subject who is deemed ineligible will be discharged from unit prior to challenge. Subjects may return for re-evaluation of challenge eligibility within 30 days of their original Day 28 Visit. If they are assessed to be eligible for challenge at that time, they may be included for challenge with a subsequent cohort.

All subjects that continue to demonstrate eligibility will fast (defined by nothing by mouth except for water), starting from the midnight prior to the challenge day.

Subjects will undergo the following assessments and procedures. Subjects will also be screened for eligibility for enrollment into the challenge phase:

- Physical examination (targeted)
- Vital signs
- ECG
- Safety Labs
    - CBC
    - Serum Chemistry
    - Urinalysis
- Female pregnancy test (urine)
- Stool sample for enteric pathogens (BioFire)
- Influenza A & B antigen test
- Drug screen (urine, alcohol breath test)
- Blood samples for immunogenicity (PBMC, serum and fixed whole blood)
- Fecal sample for shedding (pre-challenge baseline)
- Fecal sample for immunogenicity
- Saliva sample for immunogenicity
- SAM (nasal MLF evaluation)
- Review of concomitant medications
- Query for AEs

This visit is required for all subjects regardless of challenge status, since there are blood draws impacting immune endpoints from vaccination stage. It is a deviation if Visit 03 does not occur. Visit 3 has a +3 day window, followed by a +30 days window for repeat Visit 03, if needed, to rescreen subjects for challenge.

Day 28 immunogenicity samples, including stool, should be collected at the subject's original Day 28 visit and do not need to be re-collected prior to challenge, if the subject's challenge visit is delayed (+ 30 days). However, a stool sample is needed at the repeat Day 28 Visit, if challenge is postponed within window, for enteric pathogens (BioFire), if needed, and for shedding pre-challenge baseline. The stool sample for fecal immunogenicity may be collected 1 day or within 24 hours of Day 28 through pre-challenge on Day 29.

BioFire GI Testing Guidance:

- Subjects without symptoms of AGE in the 72 hours prior to Day 28 should not have BioFire GI pathogen assay
- For subjects with symptoms of AGE in the 72 hours prior to challenge who test positive on BioFire for an enteric pathogen OTHER THAN NOROVIRUS, they may be re-evaluated for a future challenge as allowed by protocol.
- If no AGE symptoms in the preceding 72 hours when evaluating for the next challenge AND the investigator considers the prior AGE fully resolved, that subject does not need a repeat BioFire GI pathogen test
- Any subject in this situation should be discussed with Vaxart MM (including which pathogen was positive on BioFire at initial challenge screening) for approval to re-evaluate for challenge.
- A subject that tests positive for Norovirus at Day 28 will be excluded from future norovirus challenge.

7.6 Visit 04: Study Day 29 (Challenge Day)

7.6.1 Pre-challenge Assessments

On the morning of challenge, fasting subjects will have baseline vitals (temperature, blood pressure, heart rate, breathing rate) recorded and a final eligibility confirmation (medical history and targeted physical examination) will be completed prior to oral ingestion of the challenge inoculum.

7.6.2 Viral Challenge Eligibility

Results of Day 28 assessments (except the immunological tests) as well as the pre-challenge assessments on Day 29, must be available and reviewed prior to virus administration. Subjects who have any of the following conditions are not eligible for participation in the challenge phase:

- clinically significant symptoms or signs of norovirus acute gastroenteritis (per the judgment of the investigator) as assessed by:
    - Investigator-assessed signs of norovirus illness
    - Targeted physical examination

  (exception: mild headache, with no signs or symptoms of infection and no fever, may be allowed);
- positive for enteric pathogens (BioFire);
- positive Influenza A or B antigen test result;
- an oral temperature of >37.5°C;
- any finding (clinical or laboratory value), that in the opinion of the investigator, should exclude the subject from viral challenge due to safety concern;
- any conditions, that in the opinion of the investigator, might interfere with the subject's ability to complete participation in the challenge (sequestration) phase, per protocol;

- any finding (clinical or laboratory value), that in the opinion of the investigator, might interfere with ability to assess the primary study objectives.

Should a subject be deemed inappropriate for continued participation into the challenge phase, the reason for ineligibility will be documented and the subject will be rolled into the safety follow-up period. The subject will enter the safety follow-up period continue to be contacted through Day 365, as described in Section 7.9 .

7.6.3 Viral Challenge Administration

Subjects will have fasted overnight prior to viral challenge. Additionally, they should have nothing by mouth, except water, for 90 minutes before and after ingestion of the challenge inoculum. Subjects will drink 100 mL of sodium bicarbonate solution (~1.3% $NaHCO_3$) prior to viral challenge; approximately 1 minute later, subjects will ingest the NV inoculum (Lot 001-09NV and Sublot 2, IND 14697) suspended in 100 mL of distilled water.

7.6.4 Visits 04 – 08: Post-Challenge Observation Period (Day 29 – Day 33 [+ 3 Days])

Following ingestion of the challenge inoculum, the following procedures will be performed:

- Study subjects have daily targeted physical exams and will be monitored for symptoms of NV illness and vital signs twice daily while in the clinical research unit post-challenge.
- All fecal samples will be graded for consistency (grade 1 to 5) and weighed.
- All emesis will be collected and weighed when available.
- Following challenge, anticipating signs and symptoms will be assessed. The maximum symptom severity for the day will be determined using a standard rating scale.
- Anticipated signs and symptoms of norovirus acute gastroenteritis include: diarrhea, vomiting, nausea, fever, abdominal cramps or pain, abdominal gurgling or bloating and myalgia. These symptoms will be solicited and graded.
- The maximum temperature, total diarrheal stool volume, number of diarrheal stools, total emesis volume, and number of emesis episodes will be calculated for each 24 h period during the inpatient stay.
- On Day 33 (either prior to discharge or if subject remains sequestered)
    - Blood sample for Immunogenicity (PBMC and fixed whole blood)
    - Safety Labs
        - CBC
        - Serum Chemistry
        - Urinalysis
    - Discharge criteria must be assessed and met (per checklist)
    - NV signs and symptoms e-Diary will be dispensed prior to discharge with instruction for use, unless subject remains an inpatient through Day 36 (Day 33 + 3days)

The nursing staff as well as the trained study staff will monitor the subject's vital signs and assess for signs and symptoms of acute NoV illness twice daily from Day 29 post challenge to discharge. if a subject experiences emesis or diarrhea after the last NV Signs and Symptoms assessment of the calendar day, the site will record the occurrence on the second Norovirus Signs and Symptoms form for the corresponding calendar day in which it occurred. If a subject experiences emesis or diarrhea after the first NV Signs and Symptoms assessment of the day but before 12:00pm, the site will record

the occurrence on the first Norovirus Signs and Symptoms for the corresponding calendar day on which it occurred. If a subject experience any of the other symptoms on the Norovirus Signs and Symptoms form after the last assessment of the calendar day, those symptoms will be recorded on the first assessment of the next day. Grading scales **(Appendices C and D)** will be used to classify diarrhea, nausea, and vomiting. Subjects will also be asked to rate symptoms of myalgia and fatigue as mild, moderate or severe. AEs and concomitant medication usage will also be collected during the inpatient stay. During the first 4 days after challenge, subjects will receive daily weight, fluid intake, and fluid output measurements. The Study Investigator, or his/her designated representative, will monitor subjects daily. The subjects will remain in the inpatient unit at least 4 days after challenge following enteric isolation procedures. All staff in contact with subjects will be asked to wear Personal Protective Equipment of at least disposable gloves, gowns, and masks.

A portion of stool and emesis samples passed by the subjects during their inpatient stay will be collected and stored at 4°C. These specimens will be tested for the presence of the NoV challenge strain by qRT-PCR. Fecal and saliva samples collected before challenge and 28 days after challenge will be tested for NoV viral protein 1 (VP1) antibody.

On the day of discharge from the inpatient unit, approximately 60 mL of whole blood will be collected for PBMC isolation, and a sample for safety labs.

7.6.4.1 Discharge Criteria

Study subjects will be evaluated for discharge 4 days after challenge (Day 33). If subject is still symptomatic with NV illness and it is clinically indicated, they will remain in the isolation unit for additional days up to Day 36. At the time of discharge, subjects will be instructed on the importance of hand washing and careful personal hygiene and given antimicrobial soap. Subjects will be asked to follow appropriate sanitation and hygiene for 1 month following virus ingestion. At the time of discharge, subjects will be given stool collection kits to take home so that they can bring a stool sample within 24 hours of their return on Day 36 (first follow-up visit). Subjects will also be dispensed an e-Diary that they will use to record signs and symptoms of NV illness daily through their Day 36 visit.

7.6.4.2 Early Termination/Early Discharge

Subjects that desire to withdraw from the study or are withdrawn by the Investigator (eg, due to continuing non-compliance or safety concerns) during the inpatient period will be given instructions on appropriate follow up. If a subject desires to leave the inpatient containment unit early, the study staff will ensure the subject is no longer having symptoms, diarrhea, or emesis. A document of early termination/withdrawal will be completed by the study staff and co-signed by the subject. The subject will not be compensated for any inpatient days that are not completed, including the day of early termination.

7.7 Visit 09: Study Day 36 (7 Days post challenge)

A targeted physical exam and vital signs will be performed. Subjects will bring in a stool collected within 24 hours of Day 36 visit. Subjects will be given stool collection kits to take home, so they can bring a stool specimen collected within 24 hours of their next follow-up visit (Day 57). Subjects will be contacted to remind them of their follow-up appointment and to bring a stool specimen with them to their next appointment. Subjects who do not bring in a stool specimen will be asked to drop off a specimen the next day. At the clinical site, stool samples will be processed per Lab Manual

Subjects who underwent challenge will undergo the following assessments and procedures:

- Vital signs
- Review of concomitant medications
- Query for AEs

- Blood samples for immunogenicity (PBMC and fixed whole blood)
- Saliva sample for immunogenicity
- SAM (nasal mucosal lining fluid)
- Symptom grading (signs of NoV infection)
- Review and collect NV signs and symptoms e-Diary

7.8 Visit 10: Study Day 57 (28 Days post challenge ± 3 days)

Subjects who underwent challenge will undergo the following assessments and procedures:

- Physical examination (targeted)
- Vital signs
- Female pregnancy test (urine)
- Blood samples for immunogenicity (serum, PBMC and fixed whole blood)
- Fecal sample for shedding
- Fecal sample for immunogenicity
- Saliva sample for immunogenicity
- SAM (nasal mucosal lining fluid)
- Review of concomitant medications
- Query for AEs

Any subjects that did not undergo viral challenge should be contacted at Day 57 for safety follow-up and queried for SAEs, AESIs and NOCIs.

For subjects who are ineligible for challenge phase, Section 7.8 of the protocol should be followed instead of Section 7.11; that is, subjects who are not challenged should begin the safety follow up at Visit 10 (Day 57). This visit may be a phone contact. No separate "early termination" visit needs to occur. Only subjects who truly terminate participation in the study should have an early termination visit.

7.9 Visit 11 (Day 120) – Visit 14 (Day 300) Safety Follow-up Contacts

- Visit 11: Study Day 120 (92 days post challenge ± 1 week)
- Visit 12: Study Day 180 (152 days post challenge ± 1 week)
- Visit 13: Study Day 240 (212 days post challenge ± 1 week)
- Visit 14: Study Day 300 (272 days post challenge ± 1 week)

Subjects will be contacted (text/email/phone) every 2 months during the safety follow-up period and queried for the following:

- Query for SAEs
- Query for AESIs and NOCIs

7.10 Visit 15: Study Day 365 (337 days post challenge ± 1 week) End of Study Contact

Subjects will be queried for the following during the Day 365 phone call: End of study contact

- Query for SAEs
- Query for AESIs and NOCIs

7.11 Early Termination

7.11.1 Early Termination (Day 1 – Day 28; Vaccine Phase)

Subjects will undergo the following assessments and procedures:

- Physical examination
- Safety labs
  - CBC
  - Serum Chemistry
  - Urinalysis
- Vital signs
- Urine pregnancy test (females)
- Fecal sample for immunogenicity
- Saliva sample for immunogenicity
- Blood sample for Immunogenicity (see **Appendix A** for details of samples and testing to be performed)
- Review and collect e-Diary if still in use prior to withdrawal
- Review of concomitant medications
- Query for AEs
- Update contact information

7.11.2 Early Termination (–ay 29 - Day 57; Challenge Phase)

Subjects who underwent challenge and terminate during the challenge period will undergo the following assessments and procedures:

- Physical examination
- Vital signs
- Urine pregnancy test (females)
- Fecal sample for immunogenicity
- Saliva sample for immunogenicity
- Blood sample for Immunogenicity (see **Appendix A** for details of samples and testing to be performed)
- SAM (nasal mucosal lining fluid)
- Review and collect e-Diary if still in use prior to withdrawal
- Review of concomitant medications
- Query for AEs
- Update contact information

7.11.3 Early Termination (Day 58 to Day 365 – Safety Follow-Up Period)

Subjects who terminate early during the safety follow up period will undergo the following assessments:

- Query for SAEs, AESIs and NOCIs

7.12 Unscheduled Visit(s)

Subjects who experience any serious or severe adverse effects or experience an event of concern can be scheduled for an additional visit for further evaluation. If an unscheduled visit occurs, a member of the clinical study team (PI, sub-investigator, nurse coordinator, or clinical nurse) will evaluate the subject to determine the cause of the visit and provide care as needed. All procedures and/or sample collections should be documented within in unscheduled visit CRF.

7.13 Immunogenicity Assessments

Primary Immunoassays

- VP1 specific IgA ASC (by MSD)
- HBGA blocking antibodies (BT_{50})
- VP1 specific serum IgG (by MSD)
- VP1 specific serum IgA (by MSD)

Exploratory Immunoassays

- VP1 specific IgG ASC
- VP1 specific IgG and IgA memory B cells (by ELISpot)
- VP1 specific IgG and IgA from plasmablast cultures (by ELISA)
- B cell immunophenotyping (by flow cytometry and/or single cell sequencing)
- Fecal VP1 specific IgA (by ELISA or MSD)
- Saliva VP1 specific IgA (by ELISA or MSD)
- Mucosal VP1 specific IgA (by ELISA or MSD)
- Fixed whole blood (by CyTOF or flow cytometry)

Additional exploratory immunogenicity assays may also be performed to further evaluate the activity of the VXA-G1.1-NN vaccine candidate on samples stored for future use (with subjects' consent). Note that not all sample timepoints may be relevant for the some of the analysis, so not all assays will be performed at all timepoints.

7.14 Assessment of Clinical Illness

Following challenge, study subjects will be monitored twice a day unless greater frequency is clinically indicated through discharge (Day 33 + 3 days) for the following gastrointestinal signs and symptoms of norovirus illness:

- diarrhea, vomiting, nausea, fever, myalgia
- abdominal cramps, pain, gurgling or bloating

7.15 Norwalk Virus Infection

NV infection as detected by qRT-PCR, > 1 positive post-challenge stool or emesis sample through day 8 post-challenge.

7.16 Microbiological Assessments

During the challenge phase sequestration period all fecal and emesis specimens will be weighed/measured for volume. All fecal and emesis will be collected daily and tested for NV. Stool will be graded. A stool sample will also be collected at Day 36 and Day 57.

8 MANAGEMENT OF EXPECTED NOROVIRUS ILLNESS

8.1 Clinical Evaluation

After NV challenge, subjects will remain in the ward for at least 4 days. Vital signs (temperature, blood pressure, heart rate, respiratory rate) will be measured twice a day (am and pm) throughout the confinement period until discharge. Subjects will be interviewed daily by a study investigator to determine the occurrence of illness signs and symptoms. This data will be recorded on a standardized form and graded in severity according to a standardized scale. A focused physical examination may be performed at the discretion of the Investigator according to the nature of a subject's complaint.

8.2 Study Definitions

Norovirus Gastroenteritis (NVG):
NVG is a composite endpoint defined as meeting one or more of the following definitions for both Acute Gastroenteritis and NV Infection during the inpatient period.

Acute Gastroenteritis (clinical NV illness):
Acute Gastroenteritis (AGE) is defined as meeting any one of the three categories listed below:

- Diarrhea:
  - ≥ 3 loose or liquid stools produced in any 24-hour period, or
  - > 400g of loose or liquid stools produced in any 24-hour period;
- Vomiting: ≥ 2 vomiting episodes in any 24-hour period, or
- Diarrhea and vomiting:
  - One vomiting episode plus any loose or liquid stool in any 24-hour period, or
  - One vomiting episode plus ≥ 2 of the following events in any 24-hour period:
    - nausea
    - fever (oral temperature ≥ 37.6°C)
    - abdominal cramps or pains
    - abdominal gurgling or bloating
    - myalgia

See **Appendix C** for grading scales for signs and symptoms of acute gastroenteritis.

Norwalk Virus Infection:

- NV infection as detected by qRT-PCR, > 1 positive post-challenge stool or emesis sample through day 8 post-challenge.

8.3 Measurement of Diarrhea and Vomitus

Since diarrhea is anticipated to be a common occurrence, all subjects will be expected to collect every stool that is passed, from the time of challenge until discharge. Subjects will be instructed to use a plastic stool collection basin (hat). Subjects will be instructed on how not to collect urine in the same container. All stools will be weighed and graded by the study staff. The grading of stool is based on consistency and the definition of diarrhea is a grade 3 or higher in stool, as follows:

- Grade 1 – well formed (does not take the shape of the container)

- Grade 2 – soft (does not take the shape of the container)
- Grade 3 – thick liquid (diarrhea, takes the shape of the container readily)
- Grade 4 – opaque watery diarrhea
- Grade 5 – rice water diarrhea (clear watery)

Any grade 3 or higher stool weight, will be used to estimate the volume of fluid loss (assume approximately 1-gram diarrheal stool = 1 mL of fluid lost).

Similarly, any episodes of vomiting should be collected in either a stool "hat" or a plastic "kidney" basin, provided. If a vomiting or diarrhea episode is not able to be collected in a basin (e.g., the subject has an "accident" while sleeping or before they are able to reach a toilet), then the volume of the output will be estimated.

8.4 Management of Fluid Loss

Subjects who develop diarrheal stools (grade 3 or higher) will begin oral fluid replacement at 1.5 times the stool volume. Vomitus will be replaced with oral fluids in equal amounts, 1:1 ratio. If a subject develops severe watery diarrhea or persistent vomiting and cannot maintain full hydration by oral means, IV fluid replacement will be administered.

Assessment of dehydration may include urine specific gravity and serum electrolytes (Sodium, Potassium, Chloride, Bicarbonate) and renal function (blood urea nitrogen [BUN] and creatinine) and physical assessment.

An Investigator is available by telephone or beeper at all times. Nurses will notify the on-call Investigator if any of the following occurs in a subject who is experiencing diarrhea and/or vomiting:

- Syncope
- Complaint of dizziness or lightheadedness or established orthostatic hypotension
- Urine specific gravity > 1.025
- > 500 mL behind in ORS replacement
- Vomiting of ≥ 500 mL once or total volume within the past 4 hours
- High fever ≥ 39°C (102.1°F)
- Severe headache, severe malaise, or severe abdominal pain
- Subject has a complaint for which he/she requests treatment
- Any other clinical situation that concerns the nurse

8.5 Indications for Concomitant Medications

Any concomitant medication, prescription or over-the-counter, will be evaluated for continuation during the inpatient setting (eg, long-standing single-agent anti-hypertensive medication or cholesterol medication). Any such medication will need to be discussed with and approved by the investigator prior to admission. The supply of medication will be the responsibility of the subject and will be handed over to the research staff upon admission; daily administration will be recorded. Any un-declared prescription or over-the-counter medications that are discovered during the inpatient stay will constitute a violation of the ward "Rules and Procedures."

Other medications may be administered during the study period as follows:

- Smoking is not allowed on the ward, but subjects will be able to request a nicotine patch.
- Anti-pyretics and analgesics (ie, ibuprofen, acetaminophen, aspirin, or similar non-steroidal agents) may be prescribed for severe headaches, other pains, or fevers (eg, sustained temperatures of ≥ 39°C).
- At the investigator's discretion (eg, upon review of serum electrolyte results during severe diarrhea), oral potassium and/or IV fluids may be administered for repletion of electrolyte losses.

- Other medications, which are deemed necessary for the safety and welfare of the subject.

The prescription of any medication must be ordered and signed by the investigator and each administration recorded.

8.6 Prohibited and Controlled Medications/Treatments

All concomitant medications will be recorded from 30 days prior to start of screening through completion of the challenge active study period (Day 57).

Subjects may not initiate new medications (or change the dose of an allowed concurrent medication) within 30 days prior to first vaccination, with exceptions, as outlined in the study exclusion criteria **Section 4.2**. Subjects will be questioned regarding any changes in concomitant medication usage through the end of the challenge active study period (Day 57). Any changes from Baseline will be recorded within the corresponding Case Report Form (CRF).

9 ASSESSMENT OF SAFETY

The safety assessment of the study vaccine and challenge virus will be through the detection and documentation of AEs, both solicited AEs and unsolicited AEs, and/or clinically significant laboratory abnormalities, from baseline (time of vaccination) through 28 days post-challenge. The occurrence of SAEs, AESIs and NOCIs will be reported through 12 months post vaccination –Day 1 - Day 365).

9.1 Definition of Adverse Event

An AE is any untoward medical occurrence in a subject after administration of the investigational vaccine and that does not necessarily have a causal relationship with the investigational vaccine. An AE can therefore be any unfavorable and unintended sign (including abnormal laboratory findings), symptoms, physical examinations, or disease temporally associated with the use of the investigational vaccine, whether related to the investigational vaccine. This definition includes exacerbations of pre-existing conditions. Stable pre-existing conditions which do not change in nature or severity during the study are not considered AEs; however, these should be reported as part of the medical history.

Solicited AEs are pre-specified reactogenicity symptoms including:

- fever
- nausea
- vomiting
- diarrhea
- headache
- malaise/fatigue
- myalgia (muscle pain)
- abdominal pain
- anorexia

9.1 Solicited Symptom Reactogenicity Table

Subjects will utilize a solicited symptom e-Diary issued on the day of vaccination to daily record the events during the first 7 days following vaccination. These solicited symptoms will be graded as follows in Table 20:

Table 20 SOLICITED SYMPTOM REACTOGENICITY TABLE

	Grading				
Symptom	**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	Event easily tolerated, causing minimal discomfort and no interference with activity	Repeated use of non-narcotic pain reliever for >24 hours or some interference with activity	Any use of narcotic pain reliever or prevents daily activity	ER visit or hospitalization
Myalgia (muscle pain)	None	Event easily tolerated, causing minimal discomfort and no interference with activity	Event sufficiently discomforting to interfere with daily activity	Significant; Prevents daily activity	ER visit or hospitalization
Abdominal Pain	None	Event easily tolerated, causing minimal discomfort and no interference with activity	Some interference with activity but no medical intervention	Prevents daily activity and requires medical intervention	ER visit or hospitalization
Anorexia	None	Event easily tolerated, causing minimal discomfort and no interference with activity	Some interference with activity but no medical intervention	Prevents daily activity and requires medical intervention	ER visit or hospitalization
Nausea	None	No interference with activity	Some interference with activity	Prevents daily activity	ER visit or hospitalization
Vomiting	None	1 or 2 episodes in 24 hours	>2 episodes in 24 hours	Requires outpatient IV hydration	ER visit or hospitalization
Diarrhea	None	2 to 3 loose stools in 24 hours	4–5 loose stools in 24 hours	6 or more loose stools in 24 hours	ER visit or hospitalization
Malaise/ Fatigue	None	Event easily tolerated, causing minimal discomfort and no interference with activity	Some interference with activity	Significant; Prevents daily activity	ER visit or hospitalization

[a] Everyday activity includes attendance at work, school, and usual habits of the subjects.

Unsolicited AEs are any AEs reported spontaneously by the subject, observed by the study personnel during study visits or those identified during review of medical records or source documents. Unsolicited AEs (all AEs not collected in the solicited symptom e-Diary during the first 7 days following vaccination) will be monitored and collected from the time of vaccination through Day 28 as AEs relevant to vaccination.

Unsolicited AEs will be monitored and collected from post challenge (Day 29) through end of active phase (Day 57) as AEs relevant to challenge virus.

9.1.2 Grading of Severity of an Adverse Event

Severity of AEs will be graded as:

Mild: events require minimal or no treatment and do not interfere with the subject's daily activities.

Moderate: events result in a low level of inconvenience or concern with the therapeutic measures. Moderate events may cause some interference with functioning and daily activities.

Severe: events interrupt a subject's usual daily activity and may require systemic drug therapy or other treatment. Severe events are usually incapacitating.

Changes in the severity of an AE should be documented to allow an assessment of the duration of the event at each level of intensity to be performed. AEs characterized as intermittent require documentation of onset and duration of each episode.

9.1.3 Relationship to Challenge Virus

Relationship (causality or attribution) of all AEs to NV infection, or to an alternate etiology (unrelated to the study) is part of the documentation process, but it is not a factor in determining what is (or is not) reported in the study. If there is any doubt as to whether a clinical observation is an AE, the event should be reported. All AEs must have their relationship assessed using 1 of 2 terms: related to NV infection, or not related (an alternate etiology can be identified and is likely or feasible). To help assess, the following guidelines are used.

Related to norovirus challenge: There is a reasonable possibility that the NV infection caused the AE. Reasonable possibility means that there is evidence to suggest a causal relationship between the NV illness and the AE, and there is no reasonable alternate etiology.

Not Related: There is not a reasonable possibility that the event is related to NV and there is a reasonable alternate etiology

9.1.4 Relationship to Study Vaccination

For all solicited and unsolicited AEs, including NOCIs, the Investigator will make a judgment regarding the relationship of the AE to the investigational product in a blinded manner. All AEs must be recorded in the source documents as well as the case report form (CRF).

The degree of certainty with which an AE is attributable to study vaccine or an alternative cause will be determined by how well the event can be understood in terms of:

- Temporal relation to administration of study vaccine administration
- The existence of other plausible explanations for the AE

The relationship of the AE to study drug administration will be specified as follows:

Not related: In the Investigator's opinion, there is no causal relationship between study drug administration and the AE;

Related: The AE follows a known temporal sequence from the time of study drug administration, cannot be explained by other disease or medications and the event is an objective and specific medical disorder or a recognized pharmacological phenomenon.

9.2 Definition of Serious Adverse Event

A SAE is any AE that results in any of the following outcomes:

1. Death
2. A life-threatening event. Life-threatening events mean that the study subject was, in the opinion of the site PI or Sponsor, at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe.
3. Requires inpatient hospitalization or prolongation of existing hospitalization
4. Results in persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
5. Congenital abnormality or birth defect
6. Important medical event that may not result in one of the above outcomes but may jeopardize the health of the study subject and/or requires medical or surgical intervention to prevent one of the outcomes listed in the above definition of SAE. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

All SAEs will be:

- recorded in the research record
- reported to the local IRB, per local IRB guidelines
- reviewed and evaluated by a study clinician and the PI
- followed through resolution by a study clinician
- All SAEs, life-threatening events and deaths, whether related or unrelated, will be reported to the study sponsor within 24 hours of site awareness.
- Other SAEs regardless of relationship, will be reported to the study sponsor within 72 hours of site awareness

9.3 Definition Adverse Events of Special Interest (AESI)

An AESI should not necessarily be classified to be a serious adverse event, even though the event may be clinically significant. If an AESI is reported, Vaxart should be promptly informed and information relevant to the event should be promptly collected using the same process as that used for reporting serious adverse events (see **Section 8.2**) Please see list of AESIs on **APPENDIX E**: Lists of Adverse Events of Special Interest.

9.4 Definition of New Onset of Chronic Illness (NOCI)

A NOCI is defined as diagnosis post study drug administration of a new medical condition, which is chronic in nature, including those potentially controllable by medication (eg, diabetes, asthma). The occurrence of NOCIs will be queried for from Day 1 post-vaccination through the safety follow-up period (Day 365).

9.5 Study Halting Rules

The study will be halted (no new enrollment, vaccinations, or challenge will be allowed pending a SMC safety review) if any of the events described below are met during the overall conduct of the study (vaccine and challenge phase).

- Two or more subjects experience a treatment related serious adverse event (SAE) of any grade, or a treatment related grade 3 or 4 AE between Day 1 and Day 28 (vaccination phase).
- Two or more subjects experience a treatment related serious adverse event (SAE) of any grade, or a treatment related grade 3 or 4 unsolicited AE within 28 days after challenge.

The SMC will provide study oversight throughout the duration of the trial interventional and safety follow-up period (Day 1 through Day 365 post-vaccination). The IRB and CBER will be notified if the study is halted for safety concerns.

9.6 Safety Oversight

A Medical Monitor (MM) will perform the oversight of safety for this study. The MM is a physician experienced in the conduct of research clinical studies. The primary responsibility of the MM is to monitor subject safety. The MM will be responsible for reviewing the cumulative safety data, including a review of safety laboratory test results and AE reporting. The MM considers study-specific data as well as relevant background information about the disease, test agent, and target population under study. The MM is empowered to request a Safety Monitoring Committee (SMC) safety review which can suspend the study, recommend amendments to the protocol, and/or to request further information.

9.7 Safety Monitoring Committee (SMC)

A SMC will be created for oversight and adjudication of AEs. The committee will consist of independent physicians with vaccine clinical trials experience, and the MM, who does not enroll patients into the study. The sponsor will have a member as a non-voting observer of the SMC. The Sponsor's Chief Medical Officer (CMO) and the study's PI will serve as a non-voting members of the SMC. The CMO's main role will be to provide background information on the study protocol as well as past safety experience with the Vaxart oral vaccine platform. The PI will provide information on study conduct. The responsibilities of the SMC will be to:

1) Review and be familiar with key clinical documentation concerning subject safety including but not limited to: study protocol, informed consent form, Investigator's Brochure and the SMC Charter.
2) Provide ongoing oversight of the study. Periodic safety reviews of the ongoing study will be conducted at pre-defined time points. Additionally, ad hoc meetings will be convened if any serious vaccine or study treatment related events or trends are observed.
3) The SMC will also have formal meetings if any of the pre-specified Halting Rules are met to review safety data and make recommendations on how to proceed. The IRB will be notified if the study is halted for safety concerns.
4) Review the symptomatic data collected for early cohorts in the challenge phase, blinded to treatment, to confirm the challenge virus is performing as anticipated may be undertaken. If fewer subjects are showing symptoms, additional interim analyses may be recommended by the SMC. Any unblinded analyses will be restricted to the SMC.
5) The SMC will function in accordance with the following provisions: (1) United States (US) Code of Federal Regulations (CFR) applicable to clinical studies (21 CFR Part 50, 21 CFR Part 54, 21

CFR Part 312.55 and 312.56. (2) ICH E6 and 62 Federal Register 25691 (1997): Good Clinical Practice (GCP): Consolidated Guideline.

Further details of the composition, pre-specified meetings and objectives of the SMC will be outlined in the SMC Charter.

10 CLINICAL MONITORING

Site monitoring is conducted to ensure that the rights and well-being of human subjects are protected, that the reported trial data are accurate, complete, and verifiable, and that the conduct of the trial follows the currently approved protocol/amendment(s), with Good Clinical Practice (GCP), and with applicable regulatory requirement(s). Monitoring refers to the methods used by sponsors of investigational studies, or Contract Research Organizations (CROs) delegated site monitoring responsibilities, to oversee the conduct of, and reporting of data from, clinical investigations. Site monitoring includes ensuring appropriate clinical investigator supervision of study site staff and third-party contractors.

Details of clinical site monitoring are documented in a Clinical Monitoring Plan (CMP). The CMP describes in detail who will conduct the monitoring, at what frequency monitoring will be done, at what level of detail monitoring will be performed, and the distribution of monitoring reports.

11 STATISTICAL CONSIDERATIONS

The primary endpoint of this study is a composite of occurrence of NVG caused by the challenge norovirus during inpatient challenge period and is defined as meeting one or more of the definitions for both acute gastroenteritis and NV infection.

11.1 Study Hypothesis

The study hypothesis is that norovirus vaccine, VXA-G1.1-NN, will protect against norovirus gastroenteritis related to norovirus infection in the challenge model. Protective efficacy (PE) will be estimated by the prevention of norovirus gastroenteritis among vaccine recipients compared to placebo recipients in those who were challenged.

The null hypothesis is that the absolute protective efficacy is zero. The two-sided alternative hypothesis is that the absolute protective efficacy is not equal to zero.

H_0: PE = 0
H_A: PE ≠ 0

The estimated protective efficacy is defined as: $\frac{AR_0 - AR_1}{AR_0}$

Where AR_1 is the observed norovirus gastroenteritis rate among vaccinated subjects and AR_0 is the norovirus gastroenteritis rate among subjects receiving placebo.

A Fisher's Exact Test with a 5% two-sided significance level will be used to evaluate the primary endpoint of clinical efficacy of VXA-G1.1-NN compared to placebo, to protect against NVG caused by the Norwalk strain challenge inoculum. The sample size will be determined in order to evaluate the primary endpoint using a Fisher's Exact test with a 5% significance level (Section 11.2)

The study hypothesis will also be evaluated using the Chi-Squared test as a sensitivity analysis for the primary endpoint as the Fisher's Exact test can be overly conservative in some circumstances.

An additional study hypothesis will also be evaluated as a sensitivity analysis. The null hypothesis will be that the absolute protective efficacy is zero, and the one-sided alternative hypothesis will be that the absolute protective efficacy is greater than zero.

H_0: PE = 0
H_A: PE > 0

This hypothesis will be evaluated using a one-sided Fisher's Exact test. The two sensitivity analyses described above will not be used for decision making for this trial; rather, these are sponsor requested corroborative analyses to aid in the interpretation of the primary analysis results and facilitate planning for potential future studies.
The formal statistical analysis plan (SAP) will provide additional guidance for interpreting results among the sensitivity analyses.

11.2 Sample Size Determination

Table 21 presents the estimated power based on different placebo attack rates (AR), various protective efficacy (PE), and a sample size of 50 per group. For example, the yellow highlighted cells suggest that: A Fisher's Exact Test with a 5% two-sided significance level will have 87.2% power to detect the difference between Control proportion of 0.4 (or AR of 40%) and Active proportion of 0.12 (or 12% or PE of 70%) when the sample size in each group is 50.

Table 21: Power Calculations

PE=50 %	Controls	Active		PE=60 %	Controls	Active		PE=70 %	Controls	Active	
	n=50	n=50	Power		n=50	n=50	Power		n=50	n=50	Power
AR				AR				AR			
30%	30%	15%	35.40%	30%	30%	12%	52.10%	30%	30%	9%	69.90%
40%	40%	20%	51.50%	40%	40%	16%	71.30%	40%	40%	12%	87.2%
50%	50%	25%	67.50%	50%	50%	20%	85.30%	50%	50%	15%	95.70%
60%	60%	30%	82.90%	60%	60%	24%	94.70%	60%	60%	18%	99%
70%	70%	35%	97.90%	70%	70%	28%	98.80%	70%	70%	21%	99%
80%	80%	40%	98.20%	80%	80%	32%	99%	80%	80%	24%	99%

Software: nQuery 8.2.1

Considering the first 19 subjects enrolled in the trial were vaccinated with a different vaccine lot than all subsequent subjects, an additional 20 subjects will be enrolled to minimize variability. Furthermore, a new sublot of the virus inoculum is needed to complete challenge of the added subjects so an additional 30 Subjects will be enrolled to reduce potential variability and mitigate unexpected results. Thus, approximately 170 subjects will be randomized and vaccinated to ensure that approximately 140 subjects are available to participate in the viral challenge 28 days post-vaccination.

11.3 Treatment Assignment Procedures

11.3.1 Randomization Procedures

Per ICH guideline E6: Good Clinical Practice (GCP), screening records will be kept at the participating site to document the reason why an individual was screened, but failed trial entry criteria. The reasons why individuals failed screening will be recorded in the Advantage eClinical (Electronic Data Capture System).

Once consented and upon entry of demographic and confirmation of eligibility for this trial, the subject will be enrolled. Subjects will be assigned randomly using permuted blocks to receive either VXA-G1.1-NN or placebo in a 1:1 ratio for up to approximately 170 subjects total.

11.3.2 Blinding Procedures

Investigators and subjects will remain blinded to treatment assignment until completion of the challenge phase (through Day 57) and validation of the clinical data. The Sponsor's clinical team and laboratory personnel will also be blinded to subject treatment through Day 57 analyses. Investigators may be unblinded prior to the final Day 365 end-of-study follow-up phone call.

Each bottle of multi-dose DP/placebo tablets will be labeled with an open label. The unblinded research pharmacist will use the randomization list to prepare the dose of DP/placebo tablets. All DP/placebo preparation and administration will be performed per study specific procedures detailed in the Study Pharmacy Manual.

Only in a medical emergency, when knowledge of the study treatment is essential for further management of the subject, will the randomization code be broken. In the event that this is necessary, the PI or designee will provide the study identification number to the research pharmacist, who will provide the PI with the treatment assignment for that subject. The PI will notify the Sponsor immediately and document the event on the appropriate study documents. If time allows, the investigator will notify the Sponsor Medical Monitor (MM) prior to unblinding the treatment received by the individual subject.

11.4 Final Analysis Plan

Clinical, safety, reactogenicity, and challenge data through approximately Day 57 (28 days post challenge) will represent the primary clinical database for this trial. Once the last subject completes the Day 57 visit, the primary clinical database will be cleaned, monitored and locked. Unblinded analyses of primary and secondary endpoints, including available safety data, are planned. As it is anticipated that subjects will remain in long-term safety follow-up at the time of these analyses, blinded site clinicians and Medical Monitors not involved in the analysis, publication or clinical study report (CSR) preparation will be responsible for assessing SAEs and AESIs, and NOCIs, until all subjects have completed the final follow-up visit. All analyses of data included in the preliminary report for early release will be considered the final analysis of these data, and also included in the final CSR.

Analysis of exploratory endpoints may be performed and released as the data are available from the research laboratories. Any such analyses would be considered the final analysis for the endpoint and included in the CSR.

The final CSR will be completed after the last subject's last visit is completed, and the final clinical database, including all long-term safety follow-up data, is cleaned, monitored and locked. Additional exploratory endpoint data not available at the time of CSR preparation may be included in an addendum to the CSR.

A formal statistical analysis plan (SAP) will be developed and finalized prior to unblinding for any interim analysis and prior the primary clinical database lock, which defines the analyses to be included in the preliminary report and final CSR.

11.5 Missing Values and Outliers

All attempts will be made to collect all data per protocol. As missing data are expected to be minimal, no imputation will be performed for missing values. Any data point that appears to be erroneous or inexplicable based on clinical judgment will be investigated as a possible outlier. If data points are identified as outliers, sensitivity analyses will be performed to examine the impact of including or excluding the outliers. Any substantive differences in these analyses will be reported.

11.6 Interim Analysis

Interim analysis (IA) will take place after about 50% of all randomized subjects have completed challenge and been discharged from the research unit. This interim analysis will reassess the appropriateness of assumptions used for the primary efficacy endpoint when the trial was designed in a blinded manner to the study team. The interim analysis data will be analyzed in an unblinded manner for the SMC to support the following recommendation rules shown in the section below.

Conditional Power assessment and Sample size re-estimation rules at IA

The sample size re-estimation rule will be set up using conditional power. Conditional power will be used to assess the futility of continuing further investigation and / or for sample size re-estimation. If the conditional power is observed to be sufficiently small at the interim analysis, we would conclude that it is futile to continue the investigation.

The sample size re-estimation rule (if necessary) will be defined using three thresholds for conditional power, denoted by c_1, c_2, and c_3. The thresholds are given by:

c_1=0.25, c_2=0.5, c_3=0.872

These thresholds define the "underpowered interval" where it is most sensible to increase the trial's sample size. The total sample size in the trial may be modified at this interim analysis as follows:

- If CP $\leq c_1$, retain the original sample size and stop the trial for futility.
- If CP $> c_1$ and CP $\leq c_2$ (unfavorable interval), premature to stop the study for futility.
- If CP $> c_2$ and CP $\leq c_3$ (underpowered interval), increase the sample size in all remaining trial arms to achieve the maximum conditional power of 87.2%.
- If CP $> c_3$, retain the original sample size and continue the trial.

The sample size re-estimation rule is non-binding and could be overridden by the sponsor or SMC. Although the interim analysis results nor SMC recommendation to continue the study required a sample size re-estimation, the sponsor decided to add 20 additional subjects to first 19 challenged subjects in a different IP lot to offset variability in vaccine lot between the first two cohorts and all others.

11.7 Exploratory Analysis

An exploratory analysis will take place after approximately 50 subjects of the planned randomized subjects have been vaccinated with the same IP lot and completed the challenge period. The results of the exploratory analysis will not impact the continuation of this study as planned, but rather, inform program development for other trials.

Additionally, a blinded interim immunogenicity analysis will be performed on Cohorts 1-9. The results of the analysis will not impact the continuation of this study as planned, but rather, inform program development for other trials.

12 SOURCE DOCUMENTS AND ACCESS TO SOURCE DATA/DOCUMENTS

12.1 Source Documentation

Source data are all information, original records of clinical findings, observations, or other activities in a clinical trial necessary for validation of the clinical data. Examples of these original documents and data records include, but are not limited to, hospital records, clinical and office charts, laboratory notes, memoranda, subjects' e-Diaries, evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate and complete, microfiches, photographic negatives, microfilm or magnetic media, x-rays, and subject files and records kept at the pharmacy, at the laboratories, and medico-technical departments involved in the clinical trial. All information on the CRF will be traceable to these source documents, which are generally maintained in the subject's study file. The source documents will also include a copy of the signed Informed Consent/ Health Insurance Portability and Accountability Act (HIPAA) authorization.

The investigator is responsible for ensuring the accuracy, completeness, legibility, and timeliness of the data reported. Source documents are maintained for recording data for each subject enrolled in this clinical study. Study subjects' data collected on the CRF during the trial will only be identified by subject number. If, as an exception, it is necessary for safety or regulatory reasons to identify the patient, both the Sponsor and the Investigator are bound to keep this information confidential.

All the information required by the protocol should be provided; any omissions require explanation. Each source document and corresponding CRF should be completed and available for monitoring and/or collection within a timely manner so that the monitor may check the entries for completeness, accuracy and legibility, ensure the CRF is signed by the Investigator, and transmit the data to the Sponsor.

All source documents and CRF should be completed in a neat, legible manner to ensure accurate interpretation of data. Black or blue ink is required to ensure clarity of reproduced copies. When making a change or correction, cross out the original entry with a single line and initial and date the change. DO NOT ERASE, OVERWRITE OR USE CORRECTION FLUID OR TAPE ON THE ORIGINAL.

The Investigator or designee must enter the information required by the protocol onto the CRF provided. The Sponsor's clinical site monitor will review the CRF for completeness and accuracy and instruct site personnel to make any required corrections or additions.

12.2 Data Management

Quality control audits of all key safety, laboratory, and clinical data in the database will be made after data entry has been completed. Coexistent medical conditions, AEs and other medical events will be coded using MedDRA dictionary. Concomitant medications will be coded using the World Health Organization Drug Dictionary (WHO-DD). When the database has been declared to be complete and accurate, the database will be locked. Any changes to the database after that time can only be made by joint written agreement of the study team.

13 QUALITY ASSURANCE AND QUALITY CONTROL

This clinical trial is internally monitored for quality assurance. A Protocol-Specific Quality Assurance Plan will be developed for this study. This plan, composed of Quality Control (QC) and Quality Assurance (QA), will be developed in collaboration with the PI, clinical study coordinator, Regulatory Affairs Specialist, and the QM Coordinator. The QC process will describe the day-to-day logic and edits checks of source documents, CRFs, and laboratory documents. The QA process involves periodic retrospective audits of study records and prospective reviews of clinical operations and assurance that all research required training has been completed as applicable. These audits also involve the review of the regulatory file, consent forms/process, eligibility, vaccine accountability and storage, specimen collection, processing, storage, and shipping. All observations are reviewed with the PI, Study

Coordinator, and Clinical Research Manager; Corrective Action Plans are formulated in response to any issue.

14 ETHICAL CONSIDERATIONS (AND INFORMED CONSENT)

14.1 Ethical Standard

The Investigator will ensure that this study is conducted in full conformity with the principles set forth in The Belmont Report: Ethical Principles and Guidelines for the Protection of Human Subjects of Research of the US National Commission for the Protection of Human Subjects of Biomedical and Behavioral Research (April 18, 1979) and codified in 45 CFR Part 46 and/or the ICH E6; 62 Federal Regulations 25691 (1997).

14.2 Institutional Review Board

The local institution must provide for the review and approval of this protocol and the associated informed consent documents by an appropriate ethics review committee or IRB. Any amendments to the protocol or consent materials must also be approved before they are placed into use unless it is in the best interest of the subjects' safety to implement changes prior to approval. In both the US and in other countries, only institutions holding a current U. S. Federal-Wide Assurance issued by the Office for Human Research Protections (OHRP) may participate.

Prior to enrollment of subjects into this clinical study, the protocol and the informed consent form(s) (ICF) will be reviewed and approved by the appropriate IRB. Any amendments to the protocol or consent materials will also be reviewed and approved by the appropriate IRB. The responsible official for the IRB will sign the IRB letter of approval of the protocol prior to the start of this clinical study. If amendments to the protocol are required, the amendments will be submitted to the IRB; an IRB letter of approval of the amendment must be obtained prior to implementing the amendment.

14.3 Informed Consent Process

Prior to consenting for the full study, subjects will initially provide informed consent for pre-screening to test for secretor status and ABO blood type. Eligible subjects will be required to sign a study specific ICF before any assessments or evaluations required by the study are performed. Informed consent will be initiated prior to the individual's agreeing to participate in the study and continues throughout the individual's study participation. The Informed Consent Form explains the storage and future use of specimens, and subjects will be asked to consent for future testing of samples. Extensive discussion of risks and possible benefits of this therapy will be provided to the subject. Consent forms describing in detail the study, study procedures and risks are given to the potential subject and written documentation of informed consent is required prior to starting any study procedure. Consent forms will be IRB-approved, and the subject will be asked to read and review the document. Upon reviewing the document, the investigator will explain the research study to the subject and answer any questions that may arise. The subjects will sign the informed consent document prior to any procedures being done specifically for the study. The subject should have the opportunity to discuss the study with their surrogates or think about it prior to agreeing to participate. The subject may withdraw consent at any time throughout the course of the trial. A copy of the informed consent document will be given to the subject for their records. The rights and welfare of the subject will be protected by emphasizing to them that the quality of their medical care will not be adversely affected if they decline to participate in this study.

14.4 Exclusion of Women, Minorities, and Children (Special Populations)

This clinical study will include women and men who are 18 years of age and older, and all minorities who meet the inclusion/exclusion criteria, regardless of religion, sex, or ethnic background.

14.5 Subject Confidentiality

Subject confidentiality is strictly held in trust by the investigators, their staff, and the Sponsor. This confidentiality is extended to cover testing of biological samples and other testing in addition to the clinical information relating to subjects.

The study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study, or the data will be released to any unauthorized third party without prior written approval of the sponsor.

The study monitors, or other authorized representatives of the sponsor may inspect all documents and records required to be maintained by the investigator, including but not limited to, medical records (office, clinic, or hospital) and pharmacy records for the subjects in this study. The clinical study site will permit access to such records.

Subject identity data will be contained in paper study records which will be kept in a locked file cabinet and in a secure electronic database, accessible only to authorized users at the clinical site. The study database will be user-restricted and password-protected. The study database will identify subjects by a coded study Volunteer ID number assigned by clinical site personnel; thus subjects will not be identified by name.

15 REFERENCES

1. Bresee JS, Widdowson MA, Monroe SS, Glass RI. Foodborne viral gastroenteritis: challenges and opportunities. Clin Infect Dis. 2002;35(6):748-53.
2. Debbink K, Lindesmith LC, Baric RS. The state of norovirus vaccines. Clin Infect Dis. 2014;58(12):1746-52.
3. Teunis PF, Moe CL, Liu P, Miller SE, Lindesmith L, Baric RS, et al. Norwalk virus: how infectious is it? J Med Virol. 2008;80(8):1468-76.
4. Marks PJ, Vipond IB, Carlisle D, Deakin D, Fey RE, Caul EO. Evidence for airborne transmission of Norwalk-like virus (NLV) in a hotel restaurant. Epidemiol Infect. 2000;124(3):481-7.
5. Graham DY, Jiang X, Tanaka T, Opekun AR, Madore HP, Estes MK. Norwalk virus infection of volunteers: new insights based on improved assays. J Infect Dis. 1994;170(1):34-43.
6. Atmar RL, Opekun AR, Gilger MA, Estes MK, Crawford SE, Neill FH, et al. Norwalk virus shedding after experimental human infection. Emerg Infect Dis. 2008;14(10):1553-7.
7. Ramani S, Neill FH, Opekun AR, Gilger MA, Graham DY, Estes MK, et al. Mucosal and Cellular Immune Responses to Norwalk Virus. J Infect Dis. 2015;212(3):397-405.
8. Glass RI, Parashar UD, Estes MK. Norovirus gastroenteritis. N Engl J Med. 2009;361(18):1776-85.
9. Vega E, Barclay L, Gregoricus N, Shirley SH, Lee D, Vinje J. Genotypic and epidemiologic trends of norovirus outbreaks in the United States, 2009 to 2013. J Clin Microbiol. 2014;52(1):147-55.
10. Vega E, Barclay L, Gregoricus N, Williams K, Lee D, Vinje J. Novel surveillance network for norovirus gastroenteritis outbreaks, United States. Emerg Infect Dis. 2011;17(8):1389-95.
11. Zheng DP, Widdowson MA, Glass RI, Vinje J. Molecular epidemiology of genogroup II-genotype 4 noroviruses in the United States between 1994 and 2006. J Clin Microbiol. 2010;48(1):168-77.

12. Debbink K, Lindesmith LC, Donaldson EF, Costantini V, Beltramello M, Corti D, et al. Emergence of new pandemic GII.4 Sydney norovirus strain correlates with escape from herd immunity. J Infect Dis. 2013;208(11):1877-87.
13. Leshem E, Wikswo M, Barclay L, Brandt E, Storm W, Salehi E, et al. Effects and clinical significance of GII.4 Sydney norovirus, United States, 2012-2013. Emerg Infect Dis. 2013;19(8):1231-8.
14. de Graaf M, van Beek J, Vennema H, Podkolzin AT, Hewitt J, Bucardo F, et al. Emergence of a novel GII.17 norovirus - End of the GII.4 era? Euro Surveill. 2015;20(26).
15. Karst SM, Wobus CE, Goodfellow IG, Green KY, Virgin HW. Advances in norovirus biology. Cell Host Microbe. 2014;15(6):668-80.
16. Vongpunsawad S, Venkataram Prasad BV, Estes MK. Norwalk Virus Minor Capsid Protein VP2 Associates within the VP1 Shell Domain. J Virol. 2013;87(9):4818-25.
17. Jiang X, Wang M, Graham DY, Estes MK. Expression, self-assembly, and antigenicity of the Norwalk virus capsid protein. J Virol. 1992;66(11):6527-32.
18. Donaldson EF, Lindesmith LC, Lobue AD, Baric RS. Viral shape-shifting: norovirus evasion of the human immune system. Nat Rev Microbiol. 2010;8(3):231-41.
19. Harrington PR, Lindesmith L, Yount B, Moe CL, Baric RS. Binding of Norwalk virus-like particles to ABH histo-blood group antigens is blocked by antisera from infected human volunteers or experimentally vaccinated mice. J Virol. 2002;76(23):12335-43.
20. Johnson PC, Mathewson JJ, DuPont HL, Greenberg HB. Multiple-challenge study of host susceptibility to Norwalk gastroenteritis in US adults. J Infect Dis. 1990;161(1):18-21.
21. Parrino TA, Schreiber DS, Trier JS, Kapikian AZ, Blacklow NR. Clinical immunity in acute gastroenteritis caused by Norwalk agent. N Engl J Med. 1977;297(2):86-9.
22. Lindesmith L, Moe C, Marionneau S, Ruvoen N, Jiang X, Lindblad L, et al. Human susceptibility and resistance to Norwalk virus infection. Nat Med. 2003;9(5):548-53.
23. Simmons K, Gambhir M, Leon J, Lopman B. Duration of immunity to norovirus gastroenteritis. Emerg Infect Dis. 2013;19(8):1260-7.
24. Tan M, Jiang X. Norovirus gastroenteritis, carbohydrate receptors, and animal models. PLoS Pathog. 2010;6(8):e1000983.
25. Ball JM, Graham DY, Opekun AR, Gilger MA, Guerrero RA, Estes MK. Recombinant Norwalk virus-like particles given orally to volunteers: phase I study. Gastroenterology. 1999;117(1):40-8.
26. Ball JM, Hardy ME, Atmar RL, Conner ME, Estes MK. Oral immunization with recombinant Norwalk virus-like particles induces a systemic and mucosal immune response in mice. J Virol. 1998;72(2):1345-53.
27. El-Kamary SS, Pasetti MF, Mendelman PM, Frey SE, Bernstein DI, Treanor JJ, et al. Adjuvanted intranasal Norwalk virus-like particle vaccine elicits antibodies and antibody-secreting cells that express homing receptors for mucosal and peripheral lymphoid tissues. J Infect Dis. 2010;202(11):1649-58.
28. Guerrero RA, Ball JM, Krater SS, Pacheco SE, Clements JD, Estes MK. Recombinant Norwalk virus-like particles administered intranasally to mice induce systemic and mucosal (fecal and vaginal) immune responses. J Virol. 2001;75(20):9713-22.

29. Tacket CO, Sztein MB, Losonsky GA, Wasserman SS, Estes MK. Humoral, mucosal, and cellular immune responses to oral Norwalk virus-like particles in volunteers. Clin Immunol. 2003;108(3):241-7.

30. Atmar RL, Bernstein DI, Harro CD, Al-Ibrahim MS, Chen WH, Ferreira J, et al. Norovirus vaccine against experimental human Norwalk Virus illness. N Engl J Med. 2011;365(23):2178-87.

31. Bernstein DI, Atmar RL, Lyon GM, Treanor JJ, Chen WH, Jiang X, et al. Norovirus Vaccine Against Experimental Human GII.4 Virus Illness: A Challenge Study in Healthy Adults. J Infect Dis. 2014;211(1):870.

32. Kim L, Liebowitz D, Lin K, Kasparek K, Pasetti MF, Garg SJ, et al. Safety and immunogenicity of an oral tablet norovirus vaccine, a phase I randomized, placebo-controlled trial. JCI Insight. 2018;3(13).

33. Liu P, Hsiao HM, Jaykus LA, Moe C. Quantification of Norwalk virus inocula: Comparison of endpoint titration and real-time reverse transcription-PCR methods. J Med Virol. 2010;82(9):1612-6.

34. Leon JS, Kingsley DH, Montes JS, Richards GP, Lyon GM, Abdulhafid GM, et al. Randomized, double-blinded clinical trial for human norovirus inactivation in oysters by high hydrostatic pressure processing. Appl Environ Microbiol. 2011;77(15):5476-82.

35. Seitz SR, Leon JS, Schwab KJ, Lyon GM, Dowd M, McDaniels M, et al. Norovirus infectivity in humans and persistence in water. Appl Environ Microbiol. 2011;77(19):6884-8.

36. Dudding BA, Bartelloni PJ, Scott RM, Top FH, Jr., Russell PK, Buescher EL. Enteric immunization with live adenovirus type 21 vaccine. I. Tests for safety, infectivity, immunogenicity, and potency in volunteers. Infection and immunity. 1972;5(3):295-9.

37. Emini EA, editor Ongoing Development and Evaluation of a Potential HIV-1 vaccine Using a Replication-Defective Adenoviral Vector. Keystone Conference on HIV Vaccines; 2003; Banff, Canada: Abstract 012.

38. Schwartz AR, Togo Y, Hornick RB. Clinical evaluation of live, oral types 1, 2, and 5 adenovirus vaccines. Am Rev Respir Dis. 1974;109(2):233-9.

39. Young LS, Searle PF, Onion D, Mautner V. Viral gene therapy strategies: from basic science to clinical application. J Pathol. 2006;208(2):299-318.

APPENDIX A: SCHEDULE OF EVENTS

VXA-NVV-201 Challenge (GI.1)	Vaccination Phase					Challenge Phase						Follow-up Phase	
Study Event	Pre-Screen	Screen	SDA	Vaccination Phase Visits		Viral Challenge	Sequestration Period			Challenge Phase Visits		Phone/Email/ Text Message	EOS Contact
Study Day	-90 to screen	-45 to -1	1	8	28	29	30	31, 32	33	36	57	120, 180 240 and 300	365 Call
Visit Number	N/A	N/A	1	2	3	4	5	6, 7	8	9	10	N/A	N/A
Windows/Compliance Range (days)	*N/A*	*N/A*	*N/A*	*0*	*+3*		*N/A*	*N/A*	*+3*	*0*	*± 3*	*± 7*	*± 7*
Informed Consent	X	X											
ABO Blood Typing	X												
Secretory Status Test (saliva)	X												
Demographics		X											
Review Eligibility Criteria		X	X		X	X							
Medical History		X	X			X							
Physical Exam, (X) = targeted exam		X	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)		
Vital Signs		X[a]	X	X	X	X	X	X	X	X	X		
ECG		X			X								
Serology (HIV, HBsAg, HCV)		X											
Safety Labs (CBC, Serum chemistry, Urinalysis)[b]		X	X[c]	X	X				X				
Blood Sample - Coagulation tests, lab panel (PT, PTT, fibrinogen)		X											
Serum/Urine Pregnancy Test[d]		X	X		X						X		
Drug Screen (urine and alcohol breath test)		X	X		X								
Prior and Concomitant medications		X	X	X	X	X	X	X	X	X	X		
Vaccination			X										
Solicited Symptoms e-Diary[e] (dispense and collect)			X	X									
Adverse Events			X	X	X	X	X	X	X	X	X	X[f]	X[f]
Challenge						X							
Inpatient stay[g]					X	X	X	X	X[g]				
Stool Sample for Enteric Pathogens (BioFire Test)					X								
Influenza A & B antigen test					X								
Collect NV Signs & Symptoms[h]						X	X	X	X	X			
Collect, Measure & Grade Stool[h]					X	X	X	X	X	X	X		
Collect and Measure (ml) Emesis[h]						X	X	X	X				
Discharge from Inpatient Stay									X				
NV Signs and Symptoms e-Diary[i] (dispense & collect)									X	X			
Immunology													
Serum antibody (BT50, IgG, IgA)			X[j,k]		X						X		
Whole blood (PBMCs)			X[j]	X	X				X	X	X		
Fixed whole blood (CyTOF)			X[j]	X	X				X	X	X		
Fecal sample (IgA)			X[j]		X						X		
Saliva sample (IgA)			X[j]	X	X					X	X		
SAM (nasal mucosal lining fluid)			X[j]	X	X					X	X		

a) Height and weight will be measured for BMI calculation

b) Safety labs to include: Complete Blood Count (CBC) Hemoglobin, Hematocrit, Platelet Count and Complete White Blood Cell Count; Chemistry panel for: ALT, AST, Total Bilirubin, BUN, Creatinine, Random Glucose, Potassium and Sodium ; and Urinalysis: glucose, protein and hemoglobin

c) Baseline safety lab tests do not need to be performed if screening safety lab tests are completed within 2 days prior to vaccination (Day 1)

d) Serum pregnancy test at Screening and urine pregnancy test at Day 1 (if more than 30 days from screening), Day 28, and Day 57

e) Solicited Symptom e-Diary: solicited symptoms of reactogenicity are collected from Day 1 – Day 8 Visit

f) SAEs, AESIs and NOCIs only

g) Inpatient period will be at least 4 days (Day 33) or until clinician determines subject is NoV symptom free, up to Day 36

h) The observation and management of norovirus illness, stool collection, measurement and grading and emesis collection and measurement are intended to be performed throughout the post-challenge inpatient period, until discharge criteria are satisfied

i) NV Signs and Symptoms e-Diary will be dispensed upon discharge from isolation unit to collect NV clinical illness data through the Day 36 Visit where it will be reviewed & collected. The e-Diary will not be dispensed if subject remains in the unit until Day 36 (Day 33 + 3 days)

j) Baseline samples collected pre-vaccination

k) 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

APPENDIX B: CHALLENGE VIRUS PREPARATION

The Norovirus GI.1 (Norwalk Virus Inoculum Lot 001-09NV, IND 14697) inoculum was prepared by [redacted] and submitted to FDA for use in clinical trials under IND 14697. The viral inoculum was prepared under Good Manufacturing Practices (GMP) conditions as described in this section. Vaxart has received authorization to cross-reference [redacted]'s IND, as well as obtained GI.1 inoculum supply from [redacted] to be utilized for viral challenge in the current GI.1 challenge protocol with the VXA-G1.1-NN vaccine. To ensure that the GI.1 inoculum remains within sterility and titer specifications, the virus lot is tested annually, and data submitted to the IND by [redacted]

Preparing Norovirus Inoculum According to cGMP

The facility that was used to manufacture the inoculum is in the [redacted] at the [redacted]. The facility consists of a processing room for producing inocula, a controlled access room for storage of released raw materials and controlled documents, a segregated lab bench to perform quality control tests, and a controlled access -80°C freezer used to store finished products. To avoid any cross contamination between products, only one product was produced at a time and the production room(s), and their associated equipment were broken down and sanitized between products. The Processing facility does not have HEPA filtered air. However, the facility generally has between Class 1000-Class 10,000 air during normal operations. All open steps were performed in a Class 100 Biosafety cabinet.

Standard Operating Procedures Used in the Facility

The facility has written Standard Operating Procedures (SOPs) that cover all operations. A list of the SOPs that are used in the facility is shown in the tables below. Manufacturing data is recorded in production batch records that are prepared for each lot of material produced. Quality control data is recorded in logbooks assigned to each quality control program. Original versions of production and quality control records are maintained in the facility and copies are provided to investigators and regulatory groups as necessary.

Table A: SOPs for Facilities and Operations

SOP No.	SOP Description
1000.01	Standard Operating Procedures and Forms
1002.01	Employee Training
1003.02	Data Entry and Error Correction
1004.01	Signature Records and Signing Authorities
1005.01	Gowning and Flow for the Clinical Core of the JVL
1006.01	Formulation Lot Number Assignment
1007.01	External Regulatory Inspections
1008.01	Shipping
1011.01	Sampling Technique
1012.01	Technology Transfer
1013.01	Validation Protocol and Report
1014.01	Personnel Hygiene
2001.02	Processing Norovirus Infected Stool & Manufacturing
2020.01	Norovirus
2032.01	Control of Raw Materials
2033.01	Clinical Core Production Room Usage

2034.01	Laminar Flow Biological Safety Cabinets
2052.01	Sample Storage and Tracking
2053.01	Raw Material Specifications
2054.01	Formulation Master Record
2505.01	Product Specification
2506.01	Batch Record for Processing Norovirus Samples
3001.01	Vialing Purified Norovirus
3002.01	Cleaning, Maintaining and Monitoring Equipment
3004.02	Preparation of Sanitation and Disinfectant Solutions
3005.01	Cleaning and Sanitizing the NPF
3006.01	Equipment Calibration
3007.01	Pipette Aids
3008.01	Operating, Maintaining and Calibrating the Met One
3009.01	RCS Air Sampler
3010.01	Equipment Identification
4005.01	Equipment Logbooks
4006.01	pH Measurement
4007.02	Environmental Monitoring for the NPF
4008.02	Determining Viral Titer of GI Norovirus by PCR
5001.01	Determining Viral Titer of GII Norovirus by PCR
5002.01	Responsibilities of Quality Assurance
5003.01	Quality Assurance Audits
5005.01	Documenting and Investigating Quality Variations
5006.01	Temporary Change Control
5007.01	Clinical Labels
5008.1	Production Lot Number Assignment
	Product Release

Quality Control/Quality Assurance

Compliance with current Good Manufacturing Practice (cGMP) is maintained through a quality systems approach. This approach involves developing specific quality control programs to address each of the regulatory requirements of GMP. The specific quality control and quality assurance programs that are used to maintain cGMP are listed below. Details on the facilities and operations control programs have been compiled in a Type V Facilities Drug Master File that will be maintained at the FDA. The investigator and other regulatory entities can access this file through letters of reference as necessary.

Table B: Facilities and Operations Controls

Controls Description
Controls over Facility and Equipment
Controls over Organization and Personnel
Sterilization and Cleaning Controls
Raw Materials Controls
Environmental Monitoring
Packaging and labeling Controls
Holding and Distribution Controls
Laboratory Testing Controls
Records, Documents and Change Controls
Corrective and Preventative Action Programs

Independent Quality Assurance Program

Drug Product Safety Testing

All products produced in the facility that will be used in humans were tested for safety using established procedures. Testing for drug safety and sterility was performed by a contract-testing lab ([redacted]) under full Good Laboratory Practice (GLP). Testing for drug purity and potency was performed in the facility using standardized procedures. The results of lot release tests were reviewed by the Laboratory Director for quality assurance, accuracy, completeness, and for any problems. Once all safety tests have been completed, a CoA was prepared for each specific lot of material. After final review, the material is then released to the investigator. A list of the safety tests that were performed and the release specifications is shown below Table C and Table D.

Table C: Lot Release Tests and Specifications (Bulk Harvest Drug Substance)

Test	Method/Laboratory	Specification	Result NV
Sterility including Bacteriostasis +Fungistasis	Immersion USP/21 CFR 610.12 [redacted] Protocol #30744,30736, and 30751	Negative	Negative
Mycoplasma	Points to consider for Viral Stocks, GLP [redacted] Protocol # 30200	Negative	Negative
Endotoxin	BET/LAL Gel Clot I/A Assay, GMP [redacted] Protocol # 30739	< 500,000 EU/mL	3640 EU/mL
In Vitro Adventitious Virus	GLP, 3 Cell Lines, 28 days [redacted] Protocol # 30625	Negative	Negative
In Vivo Virus	Adult and Suckling mice, Eggs [redacted] Protocol # 30027	Negative	Negative
Viral Titer	Quantitative RT-PCR NPF SOP #4007, #4008	>5 x 10e5 genomes/mL	5 x 10e6 genomes/mL
Identity	RT-PCR	Norovirus Strain NV	NV

Table D: Lot Release Tests and Specifications Final Fill Patient Dose Drug Product

Test	Method/Laboratory	Specification	Result NV

Sterility including Bacteriostasis +Fungistasis	Immersion USP/21 CFR 610.12 [redacted] Protocol #30744, 30751 and 30736	Negative	Negative
Mycoplasma	Points to consider for Viral Stocks, GLP [redacted] Protocol # 30200	Negative	Negative
Endotoxin	BET/LAL Gel Clot I/A Assay, GMP [redacted] Protocol # 30743	< 5000 EU/mL	235 EU/mL

APPENDIX C: GRADING OF SIGNS AND SYMPTOMS OF ACUTE GASTROENTERITIS

Grading of Stool Consistency				
normal stool		*loose or diarrheal stool*		
Grade 1	**Grade 2**	**Grade 3**	**Grade 4**	**Grade 5**
Well formed; does not take the shape of the container	Soft; does not easily take the shape of the container	Thick liquid stool; easily takes the shape of the container	Opaque watery diarrheal stool	Clear watery or "rice water" diarrheal stool

Diarrhea Severity		
Mild	**Moderate**	**Severe**
2 or more loose stools of ≥ 200gms within 48 hours *or* a single loose stool of ≥ 300gms	Cumulative loose stools of ≥ 1000gms	Cumulative loose stools of ≥ 3000gms

Symptoms	Mild Grade 1	Moderate Grade 2	Severe Grade 3	Potentially Life Threatening Grade 4
Diarrhea	(*refer to table above*)			Requires hospitalization
Vomiting (episodes/24 hour)	1-2	3-4	≥ 5	
Fever, °C (°F)	38.0 – 38.4 (100.4 – 101.1)	38.5 – 38.9 (101.3 – 102)	≥ 39 (≥ 102.2)	
Additional signs and symptoms include: nausea, abdominal cramps or pain, abdominal gurgling or bloating, myalgia	No interference with activity	Some interference with activity	Prevents daily activity	Requires hospitalization

APPENDIX D: MODIFIED VESIKARI SCALE (17 POINTS)

Duration of Diarrhea (days)	Points
1	1
2-3	2
≥ 4	3
Maximum Number of Diarrhea Stools/24 h	
1-3	1
4-5	2
≥ 6	3
Duration of Vomiting (days)	
1	1
2	2
≥ 3	3
Maximum Number of Vomiting Episodes/24 h	
0	0
1	1
2-4	2
≥ 5	3
Fever (°C)	
≤ 37	0
37.1-38.4	1
38.5-38.9	2
≥ 39	3
Dehydration	
None	0
IV treatment	2

APPENDIX E: LIST OF ADVERSE EVENTS OF SPECIAL INTEREST (AESIs)

Gastrointestinal Disorders

- Celiac disease
- Crohn's disease
- Ulcerative colitis
- Ulcerative proctitis

Liver Disorders

- Autoimmune cholangitis
- Autoimmune Hepatitis
- Primary biliary cirrhosis
- Primary sclerosing cholangitis

Metabolic Diseases

- Addison's disease
- Autoimmune thyroiditis (including Hashimoto thyroiditis)
- Diabetes mellitus type 1
- Grave's or Basedow's disease

Coagulopathy

- Acquired amegakaryocytic thrombocytopenia
- Axillary vein thrombosis
- Cerebral venous thrombosis
- Disseminated intravascular coagulation
- Hepatic vein thrombosis
- Intracranial venous sinus thrombosis
- Portal vein thrombosis
- Pulmonary thrombosis
- Severe fever with thrombocytopenia syndrome
- Thrombocytopenia
- Thrombotic thrombocytopenia purpura
- Transverse sinus thrombosis
- Vena cava thrombosis
- Amegakaryocytic thrombocytopenia
- Cavernous sinus thrombosis
- Deep vein thrombosis
- Embolism venous
- Immune thrombocytopenia
- Mesenteric vein thrombosis
- Pulmonary embolism
- Pulmonary venous thrombosis
- Subclavian vein thrombosis
- Thrombocytopenia purpura
- Thrombosis
- Vena cava embolism
- Venous thrombosis

Musculoskeletal Disorders

- Antisynthetase syndrome
- Dermatomyositis
- Juvenile chronic arthritis (including Still's disease)
- Mixed connective tissue disorder
- Polymyalgia rheumatic
- Polymyositis
- Psoriatic arthropathy
- Relapsing polychondritis
- Rheumatoid Arthritis
- Scleroderma, including diffuse systemic form and CREST Syndrome
- Spondyloarthritis, including ankylosing spondylitis, reactive arthritis (Reiter's Syndrome) and undifferentiated spondyloarthritis.
- Systemic lupus erythematous
- Systemic sclerosis

Neuroinflammatory Disorders

- Acute disseminated encephalomyelitis, including site specific variants (e.g., non-infections encephalitis, encephalomyelitis, myelitis, myeloradiculomyelitis)
- Cranial nerve disorders, including paralysis/paresis (e.g., Bell's palsy)
- Guillain-Barre syndrome, including Miller Fisher syndrome and other variants
- Immune related peripheral neuropathies and plexopathies, including chronic inflammatory demyelinating polyneuropathy, multifocal motor neuropathy and polyneuropathies associated with monoclonal gammopathy
- Multiple sclerosis
- Narcolepsy
- Optic neuritis
- Transverse Myelitis

Skin Disorders

- Alopecia areata
- Autoimmune bullous skin diseases (including pemphigus, pemphigoid and dermatitis herpetiformis
- Cutaneous lupus erythematosus
- Erythema nodosum
- Morphea
- Lichen planus
- Psoriasis
- Sweet's syndrome
- Vitiligo

Vasculitis

- Large vessels vasculitis including: giant cell arteritis such as Takayasu's arteritis and temporal arteritis
- Medium sized/and or small vessels vasculitis including: polyarthritis nodosa, Kawasaki's disease, microscopic polyangiitis, Wegener's granulomatosis, Churg-Strauss syndrome (allergic granulomatous angiitis), Buerger's disease thromboangiitis obliterans), necrotizing vasculitis and anti-neutrophil cytoplasmic antibody (ANCA) positive vasculitis (type unspecified), Henoch-Schönlein purpura, Behcet's syndrome, leukocytoclastic vasculitis.

Others

- Antiphospholipid syndrome
- Autoimmune hemolytic anemia
- Autoimmune glomerulonephritis (including IgA nephropathy, glomerulonephritis rapidly progressive, membranous glomerulonephritis, membranoproliferative glomerulonephritis, and mesangioproliferative glomerulonephritis)
- Autoimmune myocarditis/cardiomyopathy
- Autoimmune thrombocytopenia
- Goodpasture syndrome
- Idiopathic pulmonary fibrosis
- Pernicious anemia
- Raynaud's phenomenon
- Sarcoidosis
- Sjogren's syndrome
- Stevens-Johnson Syndrome
- Uveitis

APPENDIX F: Preparation of NV Challenge Inoculum

IND 14697, Amendment 31, 001-09NV Sublot 2, Manufacture and Stability and Sterility Testing Results: Preparation of NV Challenge lnoculum Work Instruction

1. PURPOSE

The purpose of this Work Instruction (WI) is to document the activities related to the dilution and preparation of an administration dose of Norwalk Virus lnoculum Lot 001-09NV sublet 2, IND 14697. This procedure may be repeated for multiple Cohorts and Subjects using completely new disposable equipment and reagents for each cohort and administration at [redacted]

2. SCOPE

The principles outlined in this WI apply to Pharmacy staff who must conduct the procedures in a Class II microbiological safety cabinet. Staff must wear appropriate personal protective equipment (PPE), including at minimum face masks, sterile gloves, gowns, and eye protection. Additionally, hair and shoe covers, will be also required. This WI will apply to the Dilution of the Stock material provided by [redacted] preparation of Retained Samples for subsequent lot release and stability/sterility testing and preparation of individual dosing vials for the current study (VXA-NVV-201).

3. ABBREVIATIONS

BSC - Biological Safety Cabinet NTF - Note to File
NV - Norwalk virus
[redacted] SWFI - Sterile Water For Injection
TMF - Trial Master File

4. MATERIALS

- PPE ([redacted] SOP# COES-105-.01)
- Sterile Glass Beaker (SO ml MFR: Pyrex)
- Sterile Glass Beaker (1000 ml MFR: Pyrex)
- Cryovials/Cryotubes (3 ml Cryovial MFR: Simport; 5 ml Cryovial MFG: VWR)
- Sciences Pipettes (1 ml Pipette MFR: Eppendorf, 5 ml Pipette MFR: PhysioCare)
- Sterile Water for Injection (SO ml vials MFR: Fresenius Kabi)
- 20 ml Syringe for Diluent (MFR: McKesson)
- 3ml Syringe Luer-Lok™Tip MFR : B-D
- Mini-Spike Vented Dispensing Pin with Luer Slip SAFSITE Valve (MFR : B Braun)

5. EQUIPMENT

- o Vortex (Vortex - VWR Analog Vortex Mixer MFR: VWR)
- o Freezer (Ultra-Low Temperature Freezer MFR: New Brunswick Model: Ul0l-86)
- o BSC (Labgard, Class II, Type A2 BSC MFR: Nuaire Model: NU-425-200)

6. WORK INSTRUCTIONS

6.1 The Pharmacist or designee will swab the outside of the tube with disinfectant and allow the frozen stock material provided by [redacted] (25-35 ml Norwalk Virus lnoculum-5.0 X 10e6 genome copies (GC)/ml, Lot 001-09NV, IND 14697) to thaw on ice in the Class II microbiological safety cabinet.

6.2 Upon observation of thawing, the Pharmacist or designee will gently Vortex the liquid material for several minutes (minimum, 5 mins.}, then:

6.2.1 Remove 6 x 1ml aliquots for virus titering. Aliquots will be shipped on dry ice for priority overnight delivery to [redacted] The remainder of the stock NV will be stored at 4 degrees Celsius (Refrigerated) until [redacted] reports titer results and commencing 6.3.

Stock titer will be quantified by qRT-PCR of three aliquots according to SOP 4007.02 ([redacted] Three additional aliquots will be reserved as back up samples.

The determined titer of 0019-09NV PBH master stock was 2.4 GC/ml, outside the established range of 5 x 10e6 gc/ml +/- 2 X 10e6 gc/ml. Therefore, the stock dilution was calculated for a final titer of 3 x 10e5 GC/ml, the current reported titer of the doses in use. Calculation of the dilution is reported in the Work Sheet for Determining the Dilution of 001-09NV PBH. A dilution of 1:8 was determined to be needed to yield 3 x 10e5 GC/ml.

Lot release testing plan ([redacted] will ship test samples directly to each Test Operator)

Test Article	Test	Test Operator	Test Code	Acceptable results	Aliquots (test and back-up)	Total number of tubes and volume
Virus stock (PBH)	qRT-PCR	UNC	SOP 4007.02	>5x10e5 GC/mL	6 x 1ml	6 x 1 ml
Lot 001-23NV	qRT-PCR	UNC	SOP 4007.02	N/A	6 x1ml at beginning of lot 6 x 1ml at mid 6 x 1ml at end	18 x 1ml
	Endotoxin	WuXi-Apptec	30518.2	<5,000 EU/mL	4 x1ml beginning of lot run 4 x 1ml end	8 x 1ml
	Sterility B/F	WuXi-Apptec	30731.1	negative	1 x 1ml at beginning of lot 1 x 1ml at end	2 x 1ml
	Sterility	WuXi-Apptec	30744.1	negative	10 x 1ml at beginning of lot 10 x 1ml at end	20 x 1ml
Total						54 x 1ml

6.3 The Pharmacist or designee will transfer 25 ml of the stock NV to a sterile 50 ml beaker and begin the 1:8 dilution of the stock material.

6.4 Transfer 12.5 ml of the stock material into a sterile 1000-ml glass beaker containing 62.5 ml SWFI and gently swirl/mix for several minutes (minimum, 5 mins), then

6.5 Transfer the remainder of the stock material to the 75 ml from 6.3 and add an additional 62.5 ml SWFI and gently swirl/mix for several minutes (minimum, 5 mins.}, then

6.6 Rinse the stock material container thoroughly into the bottle with an additional 50 ml SWFI and gently swirl/mix for several minutes (minimum, 5 mins.), finally

6.7 The total volume {200 ml, 3x10e5 GC / ml} is ready for individual pipetting of the dosing administration vials (3 ml) and of various retained samples (1 ml} to be harvested at commencement, middle and completion of aliquoting vials for the challenge dosing. All dosing and retained samples will be stored at -60C.

- Commencement - 21, 1 ml vials for sterility, endotoxin and V. titer
- Middle - 6, 1 ml vials for V. titer
- End - 21, 1 ml vials for sterility, endotoxin and V. titer

6.8 The Pharmacist/designee will then begin aliquoting the 1:8 dilution NV into the designated retained (1 ml) and "dosing" sample vials (3 ml) as described in 6.7 into the appropriate size sterile vials.

6.9 The Pharmacist/designee will prepare the vials in the following order:

6.9.1 Twenty-one (21) 1 ml samples (in 3 ml sterile cryotubes)

6.9.2 Twenty-five (25) 3 ml dosing vials (in 5 ml sterile cryotubes)

6.9.3 Six (6) 1 ml samples (in 3 ml sterile cryotubes)

6.9.4 Twenty-four (24) 3 ml dosing vials (in 5 ml sterile cryotubes}

6.9.5 Twenty-one (21) 1 ml samples (in 3 ml sterile cryotubes)

All of the 3 mL dosing vials will be held (5-60 degrees C.) until finalization of lot release testing and FDA approval of IND update.

Preparation and Administration of the Individual NV Challenge

6.10 One, 3 ml vial per subject from 6.9.2 or 6.9.4 above will be thawed (following frozen storage). The vial should be swabbed with disinfectant. Each 3 ml vial of inoculum will be added to 100 ml of distilled water in a sterile, disposable, sealable cup under sterile conditions. After mixing, the solution will look like a plastic glass of clear drinking water

6.11 The sealed cup will be transported on ice to each subject for challenge.

Since the inoculum contains live infectious human pathogen, biohazard precautions will be observed. This Includes disposing of all waste in biohazard bags, cleaning equipment and materials that have come into potential contact with the inocula with 10 % bleach solution. Cups used for administration and any leftover inocula should also be cleaned with bleach and discarded.

If the subject, person administering the dose, or the person preparing inoculum spills the inoculum, the affected area must be immediately cleaned with 10% bleach solution. Hands must be washed thoroughly with disinfectant soap and water.

Partially used vials will be disinfected and discarded and may not be used for human administration or for any in vitro experimental studies.

APPENDIX G: SUMMARY OF CHANGES TO PROTOCOL

PROTOCOL VXA-NVV-201 HISTORY :	
Document	**Date**
Protocol Amendment 5.0, Version 6.0	25 May 2023
Protocol Amendment 4, Version 5.0	07 February 2023
Protocol Amendment 3, Version 4.0	23 June 2022
Protocol Amendment 2; Version 3.0	07 January 2022
Protocol Amendment 1; Version 2.0	01 December 2021
Original Protocol; Version 1.0	28 October 2018

VXA-NVV-201 Protocol Amendment 5, Version 6.0, 25 May 2023

Protocol Amendment 5 (Version 6.0) of this phase 2 Norovirus GI.1 challenge protocol (VXA-NVV-201) was finalized in June 2022 and approved by the IRB. It is being amended to incorporate sample size increase, clarify Day 28 visit procedures, provide details on new NoV virus inoculum, update the statistical methods section to incorporate sensitivity analyses for the primary endpoint, and correct other minor typographical errors. A description of the changes along with a brief rationale for each change is presented in the table below.

Section No. & Title	Description of Change	Brief Rationale
Cover page-Product	Original text: Challenge strain: Norovirus GI.1 (Norwalk Virus Inoculum Lot 001-09NV) Updated text: (Norwalk Virus Inoculum Lot 001-09NV and Sublot 2)	Update Inoculum Lot
Administrative changes to Protocol Cover Page and footers	Updated Protocol Version	Updated sponsor new protocol version number and date.
List of Abbreviations and Acronyms	Original text: international units Updated text: infectious units	Provided intended meaning of IU
Clinical Synopsis Study Population	Original Text: 140 healthy male and female adult volunteers age 18 to 49 years inclusive with blood type O or A and who are confirmed H	Updated for actual intended sample size

	type-1 antigen secretory positive (approximately 140 subjects will be randomized and vaccinated to ensure that at least 120 subjects are available to participate in the viral challenge 28 days post-vaccination) Updated text: Approximately 170 healthy male and female adult volunteers age 18 to 49 years inclusive with blood type O or A and who are confirmed H type-1 antigen secretory positive (approximately 170 subjects will be randomized and vaccinated to ensure that approximately 140 subjects are available to participate in the viral challenge 28 days post-vaccination)	
Clinical Protocol Synopsis	Viral Challenge Inoculum Original text: Norovirus GI.1 (Norwalk Virus Inoculum Lot 001-09NV, IND 14697) Updated text: Norovirus GI.1 (Norwalk Virus Inoculum Lot 001-09NV and Sublot 2, IND 14697)	Update Challenge Inoculum
Clinical Protocol Synopsis Study design	Original text: This is a Phase 2b randomized, double-blind, placebo-controlled vaccination and challenge study to assess the protective efficacy of the Norovirus vaccine (VXA-G1.1-NN). Approximately 140 healthy adults will be randomized in a 1:1 ratio to receive one oral dose of vaccine or placebo. • Arm 1: VXA-G1.1-NN oral vaccine tablets [1x1011 IU±0.5 log]; N=70 • Arm 2: Placebo tablets similar in appearance and number to active vaccine tablets; N=70	Update Arm size, sample size and cohort increase

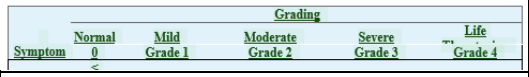
	To accommodate the limited size of the isolation unit that will be utilized for the challenge and post-challenge sequestration period, subjects will move through the study (enrollment, vaccination and challenge) sequentially in 10 to 12 cohorts. Updated text: This is a Phase 2b randomized, double-blind, placebo-controlled vaccination and challenge study to assess the protective efficacy of the Norovirus vaccine (VXA-G1.1-NN). Approximately 170 healthy adults will be randomized in a 1:1 ratio to receive one oral dose of vaccine or placebo. • Arm 1: VXA-G1.1-NN oral vaccine tablets [1x1011 IU±0.5 log]; N=85 • Arm 2: Placebo tablets similar in appearance and number to active vaccine tablets; N=85 To accommodate the limited size of the isolation unit that will be utilized for the challenge and post-challenge sequestration period, subjects will move through the study (enrollment, vaccination and challenge) sequentially in 14 to 16 Original text: Approximately 140 subjects will be dosed in the vaccination phase to ensure at least 120 subjects (~ 60 VXA-G1.1-NN vaccine and 60 placebo) are available to participate in the challenge phase.	

	Investigational Drug Product / **No. of Subjects** **Vaccination** / **140** VXA-G1.1-NN oral vaccine tablets [1x10^{11} IU±0.5 log] / 70 Placebo tablets (identical to vaccine) / 70 **Challenge** (Norwalk GI.1 Virus Inoculum) / **120** VXA-G1.1-NN oral vaccine tablets [1x10^{11} IU±0.5 log] / 60 Placebo tablets (identical to vaccine) / 60 Updated text: Approximately 170 subjects will be dosed in the vaccination phase to ensure at least 140 subjects (approximately 70 VXA-G1.1-NN vaccine and 70 placebo) are available to participate in the challenge phase. Updated table: **Investigational Drug Product** / **Approximate No. of Subjects** **Vaccination** / ~~**140**~~**170** VXA-G1.1-NN oral vaccine tablets [1x10^{11} IU±0.5 log] / ~~70~~85~~0~~ Placebo tablets (identical to vaccine) / ~~70~~85~~0~~ **Challenge** (Norwalk GI.1 Virus Inoculum) / ~~**120**~~**140** VXA-G1.1-NN oral vaccine tablets [1x10^{11} IU±0.5 log] / ~~60~~70 Placebo tablets (identical to vaccine) / ~~60~~70	
3.1 Study Design and Endpoints	Original text: This is a Phase 2b randomized, double-blind, placebo-controlled vaccination and challenge study to assess the protective efficacy of the Norovirus vaccine (VXA-G1.1-NN). To ensure at least 120 subjects (approximately 70 NV vaccine and 70 placebo) are available to participate in the challenge phase, approximately 140 healthy young adults will be randomized in a 1:1 ratio to receive one oral dose of vaccine or placebo Table 18. Each subject will receive an oral dose of 1x1011 IU±0.5 log VXA-GI.1-NN or placebo comprised of multiple tablets per dose. Updated text: This is a Phase 2b randomized, double-blind, placebo-controlled vaccination and	Update sample size

	challenge study to assess the protective efficacy of the Norovirus vaccine (VXA-G1.1-NN). To ensure at least 140 subjects (approximately 85 NV vaccine and 85 placebo) are available to participate in the challenge phase, approximately 170 healthy young adults will be randomized in a 1:1 ratio to receive one oral dose of vaccine or placebo Table 18. Each subject will receive an oral dose of 1x1011 IU±0.5 log VXA-GI.1-NN or placebo comprised of multiple tablets per dose.	
Table 10: Study Design and Vaccine Groups	Original text: VXA-G1.1-NN oral vaccine tablets [1x1011 IU±0.5 log]=70 Placebo identical to NVV=70 Challenge Norwalk Virus Strain [Lot 001-09NV (1x106 GC)]=120* Updated text: VXA-G1.1-NN oral vaccine tablets [1x1011 IU±0.5 log]=85 Placebo identical to NVV=85 Challenge Norwalk Virus Strain [Lot 001-09NV and Sublot 2 (1x106 GC)]=140	Update sample size
6.0 NV CHALLENGE INOCULUM	Added following text Updated text: To accommodate the increased sample size of the trial, additional GI.1 norovirus inoculum was diluted from the same original master stock Norwalk Virus Inoculum Lot 001-09NV purified bulk harvest (PBH), to challenge the additional Subjects. The dilution process is attached as Appendix F and an updated IND 14697 subsequently	Clarification on dilution of original master inoculum stock

	submitted to the FDA with sterility, endotoxin, and titer testing results.	
6.1 Formulation, Packaging, and Labeling of NV Challenge Virus	Added following text Updated text: The Sublot 2 inoculum material diluted and prepared for single use to complete this challenge study was packaged in one 3mL cryovials. The dilution and aliquoting of virus were performed at [redacted] in [redacted] with oversight by a representative from the original manufacturing team following the dilution procedure described in the Preparation of NV Challenge Inoculum Work Instruction, Appendix F. During the aliquoting of 001-09NV sublot 2, additional vials from the beginning, middle and end of the run were prepared for quality control testing, and shipped on dry ice to either [redacted] or [redacted] for testing.	Clarification on workflow of diluting of the original master inoculum stock
6.2 Storage and Stability of NV Challenge Virus	Added following text Updated text: The final titer for 001-09NV PBH was 2.4 x10e6 genome copies (GC)/mL. The titer in 2009 was 5 x 10e6 GC/mL. Titer of 001-09NV Final Fill sublot 1, a 1/10 dilution of 001-09NV PBH was 5 x 10e5 GC/mL in 2009 and 2.9 x 10e5 in 2022. These results indicate the virus stock has been stable for more than 10 years. Additional vials of 001-09NV Final Fill sublot 2 were tested to better evaluate the equal distribution of the virus across the dilution aliquot run. The mean of all dilutions on the standard curve that passed QC, for all nine vials across three independent assays was 5.36 10e5 GC/ml. The titer was consistent across the aliquoting run. Notably, the titer of 001-09NV sublot 1 ranged from 6 x 10e5	Updated Stability and titer information for new inoculum stock (sublot 2)

	GC/mL to 3 x 10e5 GC/mL between 2009 and 2022, indicating that 001-09NV Final Fill sublot 2 is performing consistently with 001-09NV Final Fill sublot 1. The reported titer is slightly higher than expected from the 1/8 dilution of master stock but falls within the established range of titer based on historic results of 001-09NV Final Fill sublot 1 (5 x 10e5 GC/mL +/- 2 x 10e5 GC/mL).	
7.5 Visit 03: Study Day 28, 1 day prior to challenge [+3 days for visit window and +30 days window for repeat Visit 03]	Added following text: The stool sample for fecal immunogenicity may be collected 1 day or within 24 hours of Day 28 through pre-challenge on Day 29.	Clarification of Visit 3 procedures
7.6.3 Viral Challenge Administration	Original text: Subjects will have fasted overnight prior to viral challenge. Additionally, they should have nothing by mouth, except water, for 90 minutes before and after ingestion of the challenge inoculum. Subjects will drink 100 mL of sodium bicarbonate solution (~1.3% NaHCO3) prior to viral challenge; approximately 1 minute later, subjects will ingest the NV inoculum (Lot 001-09NV, IND 14697) suspended in 100 mL of distilled water. Updated text: Subjects will have fasted overnight prior to viral challenge. Additionally, they should have nothing by mouth, except water, for 90 minutes before and after ingestion of the challenge inoculum. Subjects will drink 100 mL of sodium bicarbonate solution (~1.3% NaHCO3) prior to viral challenge; approximately 1 minute later, subjects will ingest the NV inoculum (Lot 001-09NV and Sublot 2, IND 14697) suspended in 100 mL of distilled water.	Updated inoculum lots
Definition of Adverse Event	Original text: Subjects will utilize a solicited symptom e-Diary issued on the day of vaccination to	Added language mapping solicited symptoms to the US FDA Reactogenicity grading scale

	daily record the events during the first 7 days following vaccination. Updated text: Subjects will utilize a solicited symptom e-Diary issued on the day of vaccination to daily record the events during the first 7 days following vaccination. These solicited symptoms will be graded as follows: Added Grading table 	
11.1 Study Hypothesis	Original text: The study hypothesis is that norovirus vaccine, VXA-G1.1-NN, will protect against norovirus gastroenteritis related to norovirus infection in the challenge model. Protective efficacy (PE) will be estimated by the prevention of norovirus gastroenteritis among vaccine recipients compared to placebo recipients in those who were challenged. The null hypothesis is that the absolute protective efficacy is zero. The two-sided alternative hypothesis is that the absolute protective efficacy is not equal to zero. H_0: PE = 0 H_A: PE ≠ 0 The estimated protective efficacy is defined as: $\frac{AR_0 - AR_1}{AR_0}$ Where AR_1 is the observed norovirus gastroenteritis rate among vaccinated subjects and AR_0 is the norovirus gastroenteritis rate among subjects receiving placebo. Updated text: The study hypothesis is that norovirus vaccine, VXA-G1.1-NN, will protect against	Updated to clarify that the two-sided Fisher's Exact Test will be used to evaluate the primary endpoint for the trial and to add sensitivity analyses for the primary endpoint to aid in interpretation of the primary analysis results.

	norovirus gastroenteritis related to norovirus infection in the challenge model. Protective efficacy (PE) will be estimated by the prevention of norovirus gastroenteritis among vaccine recipients compared to placebo recipients in those who were challenged. The null hypothesis is that the absolute protective efficacy is zero. The two-sided alternative hypothesis is that the absolute protective efficacy is not equal to zero. H_0: PE = 0 H_A: PE ≠ 0 The estimated protective efficacy is defined as: $\frac{AR_0 - AR_1}{AR_0}$ Where AR_1 is the observed norovirus gastroenteritis rate among vaccinated subjects and AR_0 is the norovirus gastroenteritis rate among subjects receiving placebo. A Fisher's Exact Test with a 5% two-sided significance level will be used to evaluate the primary endpoint of clinical efficacy of VXA-G1.1-NN compared to placebo, to protect against NVG caused by the Norwalk strain challenge inoculum. The sample size will be determined in order to evaluate the primary endpoint using a Fisher's Exact test with a 5% significance level (Section 11.2) The study hypothesis will also be evaluated using the Chi-Squared test as a sensitivity analysis for the primary endpoint as the Fisher's Exact test can be overly conservative in some circumstances. An additional study hypothesis will also be evaluated as a sensitivity analysis. The null hypothesis will be that the absolute protective efficacy is zero, and the one-sided alternative hypothesis will be that the absolute protective efficacy is greater than zero. H_0: PE = 0	

	H_A: PE > 0 This hypothesis will be evaluated using a one-sided Fisher's Exact test. The two sensitivity analyses described above will not be used for decision making for this trial; rather, these are sponsor-requested corroborative analyses to aid in the interpretation of the primary analysis results and facilitate planning for potential future studies. The formal statistical analysis plan (SAP) will provide additional guidance for interpreting results among the sensitivity analyses.	
11.2 Sample Size Determination	Original text: Considering the first 19 subjects enrolled in the trial were vaccinated with a different vaccine lot than all subsequent subjects, an additional 20 subjects will be enrolled to minimize variability. Thus, approximately140 subjects will be randomized and vaccinated to ensure that at least 120 subjects are available to participate in the viral challenge 28 days post-vaccination. Updated text: Considering the first 19 subjects enrolled in the trial were vaccinated with a different vaccine lot than all subsequent subjects, an additional 30 subjects will be enrolled to minimize variability. Furthermore, a new sublot of the virus inoculum is needed to complete challenge of the added subjects so an additional 20 Subjects will be enrolled to reduce potential variability and mitigate unexpected results. Thus, approximately170 subjects will be randomized and vaccinated to ensure that approximately 140 subjects are available to participate in the viral challenge 28 days post-vaccination. The increased sample size may also facilitate determining an immunologic correlate of protection.	Updated statistical methodology, power calculations and sample size

Appendix F: Preparation of NV Challenge Inoculum	See updated text in Appendix F	Added Work instruction for preparation of NV Challenge Inoculum
SUMMARY OF CHANGES TO PROTOCOL	Renamed the Table of Amendments to Appendix G	Clarification

VXA-NVV-201 Protocol Amendment 4, Version 5.0, 07 February 2023

Protocol Amendment 4 (Version 5.0) of this phase 2 Norovirus GI.1 challenge protocol (VXA-NVV-201) was finalized in June 2022 and approved by the IRB. It is being amended to incorporate sample size increase, addition of an exploratory analysis after approximately 50 Subjects complete the challenge period and being vaccinated under the same lot, adding a blinded interim immunogenicity analysis for the same subjects included in the planned interim analysis, as well as other administrative changes for clarity. A description of the changes along with a brief rationale for each change is presented in the table below.

<table>
<tr><th>Section No. & Title</th><th>Description of Change</th><th>Brief Rationale</th></tr>
<tr><td>Administrative changes to Protocol Cover Page</td><td>Updated Protocol Version</td><td>Updated sponsor new protocol version number and date.</td></tr>
<tr><td>Clinical Protocol Synopsis; Study Design</td><td>Updated Study Population by increasing the number of healthy volunteers</td><td>Increased the number of healthy Volunteers from 100 to 140. Count of Randomized and Vaccinated subjects increased from 120 to 140 to ensure that at least 120 subjects are available to participate in the viral challenge 28 days post-vaccination</td></tr>
<tr><td>Clinical Protocol Synopsis; Study Design.</td><td>Updated the study Design and number of subjects in the table.</td><td>Approximately 140 subjects will be dosed in the vaccination phase to ensure at least 120 subjects (~ 60 VXA-G1.1-NN vaccine and 60 placebo) are available to participate in the challenge phase.
<table>
<tr><th>Investigational Drug Product</th><th>No. of Subjects</th></tr>
<tr><td>Vaccination</td><td>140</td></tr>
<tr><td>VXA-G1.1-NN oral vaccine tablets
[1x10^{11} IU±0.5 log]</td><td>70</td></tr>
<tr><td>Placebo tablets (identical to vaccine)</td><td>70</td></tr>
<tr><td>Challenge (Norwalk GI.1 Virus Inoculum)</td><td>120</td></tr>
<tr><td>VXA-G1.1-NN oral vaccine tablets
[1x10^{11} IU±0.5 log]</td><td>60</td></tr>
<tr><td>Placebo tablets (identical to vaccine)</td><td>60</td></tr>
</table>
</td></tr>
</table>

Clinical Protocol Synopsis; Statistical Considerations	Updated the statistical considerations for Exploratory Analysis and added an additional blinded interim immunogenicity Analysis.	An exploratory analyses to be performed after approximately 50 subjects of the planned randomized subjects that have been vaccinated with the same IP lot and completed the challenge period. The results of the exploratory analyses will not impact the continuation of this study as planned, but rather, inform program development for other trials. Additionally, a blinded interim immunogenicity analysis will be performed on Cohorts 1-5 and shared with the sponsor. The results of the analyses will not impact the continuation of this study as planned, but rather, inform program development for other trials.
Section 7 STUDY PROCEDURE AND SCHEDULE. 7.5	Updated the section Name 7.5 'Visit 03: Study Day 28, 1 day prior to challenge [+3 days for visit window and +30 days window for repeat Visit 03. Also Removed Note.	Updated the Section Name from "**Visit 03: Study Day 28 [+30 days], 1 day prior to challenge" to** "Visit 03: Study Day 28, 1 day prior to challenge [+3 days for visit window and +30 days window for repeat Visit 03] * Removed - Note: Day 28 immunogenicity samples should be collected at the subject's original Day 28 visit and do not need to be re-collected prior to challenge, if the subject's challenge visit is delayed (+ 30 days).
Section 7 STUDY PROCEDURE AND SCHEDULE. 7.5	Added Additional Notes and specific guidance on BioFire GI Guidance	The visit is required for all subjects regardless of challenge status, since there are blood draws impacting immune endpoints from vaccination stage. It is a deviation if Visit 03 does not occur. Day 28 immunogenicity samples should be collected at the subject's original Day 28 visit and do not need to be re-collected prior to challenge, if the subject's challenge visit is delayed (+ 30 days). Visit 3 has a +3 day window, followed by a +30 days window for repeat Visit 03, if needed, to rescreen subjects for challenge. BioFire GI Testing Guidance: • Subjects without symptoms of AGE in the 72 hours prior to Day 28 should not have BioFire GI pathogen assay • For subjects with symptoms of AGE in the 72 hours prior to challenge who test positive on BioFire for an enteric pathogen OTHER THAN NOROVIRUS, they

		may be re-evaluated for a future challenge as allowed by protocol. • If no AGE symptoms in the preceding 72 hours when evaluating for the next challenge AND the investigator considers the prior AGE fully resolved, that subject does not need a repeat BioFire GI pathogen test • Any subject in this situation should be discussed with Vaxart MM (including which pathogen was positive on BioFire at initial challenge screening) for approval to re-evaluate for challenge. • A subject that tests positive for Norovirus at Day 28 will be excluded from future norovirus challenge.
Section 7.6.4: Visits 04 – 08: Post-Challenge Observation Period (Day 29 – Day 33 [+ 3 Days])	Added additional instructions related to Emesis or Diarrhea occurred after the Last NV signs and symptoms and first NV symptoms of the day but before 12:00Pm.	. If a subject experiences emesis or diarrhea after the first NV Signs and Symptoms assessment of the day but before 12:00pm, the site will record the occurrence on the first Norovirus Signs and Symptoms for the corresponding calendar day on which it occurred. If a subject experience any of the other symptoms on the Norovirus Signs and Symptoms form after the last assessment of the calendar day, those symptoms will be recorded on the first assessment of the next day
Section 7.8: Visit 10: Study Day 57 (28 Days post challenge ± 3 days)	Added additional guidance for the subjects who are ineligible for challenge phase.	For subjects who are ineligible for challenge phase, Section 7.8 of the protocol should be followed instead of Section 7.11; that is, subjects who are not challenged should begin the safety follow up at Visit 10 (Day 57). This visit may be a phone contact. No separate "early termination" visit needs to occur. Only subjects who truly terminate participation in the study should have an early termination visit.
Section 7.9: Visit 11 (Day 120) – Visit 14 (Day 300) Safety Follow-up Contacts	Added the number of days expected for each visit	• Visit 11: Study Day 120 (92 days post challenge $\pm$ 1 week) • Visit 12: Study Day 180 (152 days post challenge $\pm$ 1 week) • Visit 13: Study Day 240 (212 days post challenge $\pm$ 1 week) • Visit 14: Study Day 300 (272 days post challenge $\pm$ 1 week)
Section 7.10: Visit 15: Study Day 365 (337 days post challenge + 1 week) End of Study Contact	Added Number of days to the name of the visit	Updated the section Name from "Visit 15: Study Day 365 End of Study Contact" to "Visit 15: Study Day 365 (337 days post challenge + 1 week) End of Study Contact

Section 7.11: Early Termination	Removed wordings in section 7.11.2 Early Termination (Day 29 - Day 57; Challenge Phase)	***REMOVED *** Subjects who did not undergo challenge and discontinue between Days 29 and Day 57 do not need the above listed ET assessments, but rather should be managed as early terminations during the Safety Follow-up Period (see Section 7.11.3).
Section 11: STATISTICAL CONSIDERATIONS;	Updated the considerations for table 20 in section 11.2. Sample size determination	Considering the first 2 cohorts of the trial were vaccinated with a different IP lot than in all subsequent cohorts, it was decided to add 20 additional subjects to first 19 subjects challenged in a different IP lot into the trial. Thus, approximately 170 subjects will be randomized and vaccinated to ensure that at least 140 subjects are available to participate in the viral challenge 28 days post-vaccination.
Section 11.6: Interim Analysis.	Updated the section related to interim analysis.	Although the interim analysis results nor SMC recommendation to continue the study required a sample size re-estimation, Vaxart decided to add 20 additional subjects to first 19 challenged subjects in a different IP lot to offset variability in vaccine lot between the first two cohorts and all others.
Section 11.7: Exploratory Analysis	Updated the details related to exploratory Analysis and an additional blinded interim immunogenicity Analysis.	An exploratory analysis will take place after approximately 50 subjects of the planned randomized subjects have been vaccinated with the same IP lot and completed the challenge period. The results of the exploratory analysis will not impact the continuation of this study as planned, but rather, inform program development for other trials. Additionally, a blinded interim immunogenicity analysis will be performed on Cohorts 1-5. The results of the analysis will not impact the continuation of this study as planned, but rather, inform program development for other trials.

VXA-NVV-201 Protocol Amendment 3, Version 4.0, 23 June 2022

Protocol Amendment 2 (Version 3.0) of this phase 2 Norovirus GI.1 challenge protocol (VXA-NVV-201) was finalized in January 2022, and was approved by the IRB. Study enrollment and challenge for the first 2 cohorts was conducted under Amendment 2. The protocol is being amended to incorporate changes to the study drug section as subsequent cohorts will be dosed with drug product lots that allow administration via a single dose of tablets (rather than three sub-doses). Additionally, information to support an interim analysis following completion of challenge in ~50 subjects (50%) has been incorporated into the protocol. Minor changes to the sample collection schedule, as well as the Sponsor and Clinical Site study teams have been incorporated within Protocol Amendment 3 (Version 4.0). A description of the changes along with a brief rationale for each change is presented in the table below.

Section No. & Title	Description of Change	Brief Rationale
Administrative changes to Protocol Cover Page	Updated Contact Information and Protocol Version	Updated sponsor and site contact and new protocol version number and date.
Synopsis: Study Design Section 7.14 Assessment of Clinical Illness	Post Vaccination, change in the frequency of collection of NVV Signs and symptoms during the inpatient period	The frequency of collection of NVV signs and symptoms post challenge has been modified for optimal data collection from every 2 hours during the first 48 hours to twice a day (am and pm) for the duration of the post challenge sequestration period.
Synopsis: Study Visits: Challenge Phase Section 8 Clinical Evaluation:	Updated immunogenicity and safety assessments	Evaluation of immune response and safety assessment were added on Day 33 and 57 of challenge phase to allow for a more complete immune response dataset.
Synopsis: Safety, Immunogenicity and Microbiological Assessments	Updated frequency of collection of vital signs collection	The frequency of collection of vital signs has been modified from every 2 hours for the first 48 hours to twice a day (am and pm) during the post-vaccination phase until discharge. This approach allows for adequate data collection while balancing the burden on study subjects and site staff.
Synopsis: Study Endpoint; Section 3.2.4. Immunogenicity Endpoints :	Updated exploratory endpoints	Saliva and nasal samples collection was added on Day 8 to allow for a more complete immune response dataset.
Synopsis: Statistical Considerations Section 11: Statistical Considerations	Study hypothesis updated and details added regarding planned interim analysis	Information added in support of the Interim Analysis.
Section 5.1.3 Drug Product Dose Preparation and Administration	Updated Study drug administration information and added reference to pharmacy manual	Section was updated to remove language on sub-doses as new lot of DP to be utilized to dose remaining cohorts will utilize a single dose approach; reference to pharmacy manual was added.
Section 7.4 Visit 02: Study Day 8	Added saliva and nasal samples collection	To evaluate presence of NoV viral protein (VP1) antibody on Day 8, saliva and nasal samples collections were added.
Section 7.6. Visits 04 – 08: Post Challenge Observation Period (Day 29 – Day 33 [+ 3 Days])	Updated frequency of collection of vital signs and NVV signs and symptoms collection of immunogenicity blood samples	To allow for more accurate and timely collection of NVV signs and symptoms for analysis, frequency of collection of signs and symptoms was changed from every 2 hours to twice a day (am and pm). Fixed whole blood sample collection was added to be collected on Day 33 to evaluate further immunogenicity during the challenge phase.

Section 7.13 Immunogenicity Assessment	Updated Immunogenicity Assessment	Removed Ad5 neutralizing antibody and Nab responses by norovirus enterocyte culture assay (optional)

VXA-NVV-201 Protocol Amendment 2 (Version. 3.0), 07 January 2022

Overall Rationale for the Amendment:

Protocol Amendment 1 (Version 2.0) of this phase 2 Norovirus GI.1 challenge protocol (VXA-NVV-201) was finalized in December 2021, was approved by the IRB and submitted for review. However, during study set-up areas for modification were identified. These included the need to add testing for influenza A & B prior to viral challenge and to clarify that subjects must be negative for enteric pathogens (via BioFire) as well as influenza prior to challenge. Additionally, to ensure more accurate and timely collection of solicited symptoms of reactogenicity post vaccination and signs and symptoms of norovirus illness post viral challenge the use of an e-Diary (rather than paper diary card) is being implemented in Amendment 2. Lastly, the initial drug product lot of VXA-G1.1-NN vaccine tablets to be utilized for dosing in this study has a concentration of $2.2x10^9$ IU per tablet. Therefore, to deliver the full clinical dose of $1x10^{11}$ IU±0.5 log ~45 tablets will need to be administered. To accommodate delivery of the high number of tablets, dosing at Day 1 has been divided into 3 sub-doses of 15 tablets per sub-dose, administered 2 hours apart over a 4-hour period. In addition to the changes described above, a few minor modifications for added consistency and clarify have also been incorporated into Amendment 2 (Version 3.0).

The table below summarizes the changes incorporated into Amendment 2 and provides a brief rationale for each modification.

Section No. & Title	Description of Change	Brief Rationale
List of Abbreviations and Acronyms	Additions of e-Diary	Updated with new terms utilized in Amendment 2 for sake of completeness.
Globally throughout Protocol	Reference to diary cards has been modified to e-Dairy (electronic diary)	To allow for more accurate and timely collection of solicited symptoms of reactogenicity post vaccination and signs & symptoms of norovirus illness post viral challenge, e-Diary will be used instead of paper diary cards.
Synopsis 4.1 Subject Inclusion Criteria	Inclusion Criteria # 9: Italicized text added Male subjects must agree not to father a child or donate sperm, *as well as to use contraception/barrier (a male condom) or be abstinent from heterosexual intercourse,* from vaccination through the active period (Day 57)	The inclusion criteria regarding male birth control and contraception was expanded for added clarification.

Section 1.6: Table 14	Table 14: Description of illness after challenge with two different doses of Norwalk virus (Study VXA-G11-201.1)	The formatting for table 14 for corrected. No changes were incorporated to the data presented.
Section 5.1.3: Drug Product Dosage Preparation and Administration Section 7.3: Visit 01: Study Day 1, Study Drug Administration	Preparation of Drug Product: the following paragraph was added: The initial lot of VXA-G1.1-NN DP [Lot: DP-05.21001] that will be utilized for clinical dosing in this protocol has a concentration of $2.2x10^9$ IU per tablet. Therefore, multiple tablets (n=45) of the DP will be dispensed per dose to deliver the complete investigational dose of $1x10^{11}$ IU ± 0.5 logs in the active treatment group. To maintain the study blind, the same number of placebo tablets (n=45) will be dispensed to subjects in the control group. **Study Drug Administration** – additional information was added as shown below: Study subjects should be fasting and refrain from ingesting solid food for at least 4 hours prior *to initiation* of oral dosing. Following randomization subjects will be dispensed their assigned DP dose (VXA-G1.1-NN or placebo tablets) on Day 1. *Dosing will be divided into three sub-dosing periods: Time = 0 (dose 1/3), Time = 2 hours (dose 2/3) and Time = 4 hours (dose 3/3). At each of the 3 sub-dosing periods 15 tablets will be dispensed, which subjects will swallow with 360 to 480 mL of water. After each sub-dose subjects will be dispensed a light snack (e.g., crackers) to aid in tablet transit out of the stomach. A total of 45 tablets will be dispensed to deliver a full vaccine dose of $1x10^{11}$ IU ± 0.5 logs or a matching placebo dose.* Normal food consumption may resume 90 minutes after dosing.	The VXA-G1.1-NN vaccine DP [Lot# DP-05-25001] that will supply dosing in this study has a per tablet concentration of $2.2x10^9$ IU. Therefore, to deliver the full clinical dose of $1x10^{11}$ IU±0.5 log ~45 tablets will need to be administered. To accommodate delivery of the high number of tablets and to ensure adequate absorption in the small intestines, dosing at Day 1 has been divided into 3 sub-doses of 15 tablets per sub-dose, administered 2 hours apart over a 4-hour period Information on study drug administration procedures with sub-dosing over 4 hours was added.
Section 7.2 Visit 00: Screening Visit (Study Day -45 to -1 Appendix A : Schedule of Events	The Blood sample for BT_{50} titer was removed from the screening assessments	This test was within this section in error. No immunogenicity samples are being collected prior to Day 1.
Section 7.3 Visit 01: Study Day 1, Study	A note was added indicating the following: *(Note: Safety labs do not need to be performed if screening safety lab tests were completed within*	Modification was incorporated stating that if the safety labs for the screening visit were collected within 2 days of the

Drug Administration Appendix A: Schedule of Events	*2 days prior to study drug administration on Day 1)*	baseline visit at Day 1, then the screening values can be utilized for Day 1 safety assessments and do not need to be repeated.
Section 7.5: Visit 03: Study Day 28 [+30 days], 1 day prior to challenge	The following note has been added: *Note: Day 28 immunogenicity samples should be collected at the subject's original Day 28 visit and do not need to be re-collected prior to challenge, if the subject's challenge visit is delayed (+ 30 days).*	Note added to clarify that Day 28 immunogenicity samples should be collected at the scheduled visit (4 weeks posts vaccination), even if the subject's challenge visit is delayed (+ 30 days), to allow consistency across all subject data for this timepoint.
Section 7.5: Visit 03: Study Day 28 [+30 days], 1 day prior to challenge Appendix A: Schedule of Events	Influenza A & B antigen test has been added to the pre-challenge assessments at Day 28 (+ 30 days)	Screening for influenza A & B has been added pre-challenge to ensure that subjects infected with flu are not participating in the viral challenge. Screening out these subjects will ensure that infectious subjects are not being admitted to the clinical isolation unit, and to help ensure that signs & symptoms associated with flu are not being interpreted as associated with NV illness.
Section 7.6.2 : Viral Challenge Eligibility	The following to criteria added to the list for viral challenge eligibility: • *positive for enteric pathogens (BioFire)* • *positive Influenza A or B antigen test result*	Screening out subjects positive for enteric pathogens and influenza will ensure that infectious subjects are not being admitted to the clinical isolation unit, and to help ensure that assessments of signs & symptoms of NV illness are not confounded due to preexisting infection.
Section 15: References	Section was moved to be presented directly following Section 14.	Moved to remain with the body of the protocol rather than following the Appendices. No change to content was incorporated.

Additional minor wording, spelling and/or formatting modifications have been incorporated for added clarity and consistency.

VXA-NVV-201 Protocol Amendment 1 (Version. 2.0), 01 December 2021

Overall Rationale for the Amendment:

The original version of this phase 2 Norovirus GI.1 challenge protocol (VXA-NVV-201) was finalized in October 2018 and submitted for CBER and IRB review. Although approvals were obtained, subject enrollment was not initiated due to insufficient supply of investigational drug product (VXA-G1.1-NN vaccine) at the time. The study is being reinitiated at this time utilizing the same sample size and study design as the original protocol. Updates to the background and clinical experience sections, as well as the Sponsor and Clinical Site location and study teams have been incorporated within Protocol Amendment 1. Additionally, safety and risk information has been updated to incorporate clinical experience with the Vaxart oral vaccine platform as well as regulatory input obtained over the past few years.

The table below summarizes the changes incorporated into Amendment 1 as well as a brief rationale for each modification.

Section No. & Title	Description of Change	Brief Rationale
Title Page	Vaxart's address and study team contact information has been updated. The Clinical site's address and contact information has been updated to reflect new contact.	The title page has been updated to reflect current information on the Sponsor and clinical site.
List of Abbreviations and Acronyms	Additions of CyTOF, Nab, PF4 and TTS	Updated with new terms utilized in Amendment 1 for sake of completeness.
Synopsis 6.3 Preparation and Administration of NV Challenge Virus	Information on the Viral inoculum illness/infection rate has been updated based on the G1.1 titration study completed in 2019 (Mateo, et al). Original 60%- 70%- changed to 50%-65%	Updated to reflect most current information.
Synopsis	Cohort sizes have been updated based on [redacted] new isolation ward capacity	The size of the subject cohorts has been reduced to accommodate the size of the clinical isolation unit which has 10 subject rooms.
Synopsis 4.2 Subject Exclusion Criteria	Exclusion Criteria: An exclusion criterion has been added to exclude subjects at risk for clotting events and thrombocytopenia #4 and #5 4. *Laboratory values outside the range of normal for platelet counts and the following coagulation tests: PT/INR, aPTT and fibrinogen* 5. *Any of the following history or conditions that may lead to higher risk of clotting events and/or thrombocytopenia:*	Per guidance received from FDA, subjects will be screened for risk factors of clotting disorders prior to dosing and excluded if they have laboratory values or family history that places them at higher risk of thrombocytopenia and thrombosis syndrome (TTS), that has been reported with some injected Ad5 vectored vaccines for COVID-19.

	i. Family or personal history of bleeding or thrombosis *j. History of heparin-related thrombotic events, and/or receiving heparin treatments* *k. History of autoimmune or inflammatory disease* *l. Presence of any of the following conditions known to increase risk of thrombosis within 6 months prior to screening:* ▪ *Recent surgery other than removal/biopsy of cutaneous lesions* ▪ *Immobility (confined to bed or wheelchair for 3 or more successive days)* ▪ *Head trauma with loss of consciousness or documented brain injury* ▪ *Receipt of anticoagulants for prophylaxis of thrombosis* ▪ *Recent clinically significant infection*	(See Section 2.1: Potential Risks and Benefits).
4.2 Subject Exclusion Criteria	Exclusion Criterion #6: ECG findings within 30 days was changed to 45 days	The evaluation period for ECG was modified to match the protocol screening period.
4.2 Subject Exclusion Criteria	Exclusion Criterion #14: Added in clarification for Malabsorption to include e.g. Celiac disease, gluten intolerance	Additional information added for sake of clarification.
4.2 Subject Exclusion Criteria	Exclusion Criterion #24: Removed stool sample with occult blood during screening and added in its place, *History if hematochezia (blood in stool) or melena (black stool).*	Vaxart has dosed ~600 adult subjects up to age 80 years with its oral Ad5 vaccines. A safety signal (solicited and/or unsolicited AEs) related to GI bleeding has not been observed. Hence to facilitate the screening process, the test for occult blood at screening has been removed. However, a new exclusion criterion which specifically excludes individuals with any history of GI bleed has been added.
4.2 Subject Exclusion Criteria	Exclusion Criterion #29: Added clarification for the drug test positive for marijuana; concurrent or ongoing use of marijuana during the active study period.	Additional information added for sake of clarification.
Synopsis	Within Safety, Immunogenicity and Microbiological Safety Assessments: Solicited symptoms of reactogenicity. Myalgia (Muscle pain) was added	Inadvertently left out of the original protocol

Synopsis 3.2.4 Immunogenicity Endpoints 7.13 Immunogenicity Assessments	Within Safety, Immunogenicity and Microbiological Assessments and Study Endpoints: VP1 specific serum IgA (by MSD) was added into the Primary Immunoassays and removed from Exploratory Immunoassays. Both the VP1 Specific serum IgA and IgG will be run by MSD instead of ELISA	Updates incorporated based on advancements in assay development achieved by Vaxart over past few years. The VP1 specific serum IgA (by MSD) will be qualified, hence elevated to a primary assay in the protocol.
Synopsis 3.2.4 Immunogenicity Endpoint 7.2 Visit 00: Screening Visit	Additional information added for safety laboratory panels	Additional information added for sake of clarification.
1.4 Summary of Clinical Studies with Vaxart Oral Vaccine Platform	Section updated to incorporate safety and immunogenicity information on clinical trials completed in last several years.	Modified to include updated information.
1.4.3 Clinical Study VXA-NVV-103 with VXA-G1.1-NN and VXA-G2.4-NS	Additional information on the VXA-NVV-103 was added. Including: • Overall Study Design and Plan • Table 8: Design of Study VXA-NVV-103 • Summary of safety • Table 9: Summary of Solicited symptoms of reactogenicity • Summary of Immunogenicity Analysis	Modified to include updated information.
1.6 NV Challenge Dose Titration Study and Dose Justification	Additional information from the VXA-G11-201.1 Study: • Table 14: Description of illness after challenge with two different doses of Norwalk virus (Study VXA-G11-201.1). Was updated with all compiled data • Clinical outcome was added • Table 15: Summary of the correlation between acute gastroenteritis and NV positivity by cohort. Added • Table 16: Characterization of diarrhea in subjects with acute gastroenteritis and NV positivity by cohort (by mean). Added	Modified to include updated information.
1.7 Summary of Clinical Experience with Vaxart Oral Vaccine Platform	Summary of Clinical Experience with Vaxart Oral Vaccine Platform section and Table 7 where updated to include recent completed studies.	Modified to include updated information.

1.9 Potential Risks and Benefits	Additional of: *"Since the initiation of the current protocol, multiple injected adenovirus vectored vaccines approved for use via Emergency Use Authorization (EUA) for the prevention of COVID-19 have reported severe events of thrombosis in combination with thrombocytopenia (thrombosis with thrombocytopenia syndrome, TTS), in some cases accompanied by bleeding. Though the TTS events were reported with adenovirus vectored vaccines for COVID-19, the FDA has asked Sponsors of adenovirus vectored vaccines for other indications to broadly monitor for these risks.* *Investigators should be alert to the signs and symptoms of thromboembolism and/or thrombocytopenia. Study participants should be instructed to seek immediate medical attention if they develop symptoms including, but not limited to, shortness of breath, chest pain, leg pain and/or swelling, persistent abdominal pain, severe or persistent headaches, blurred vision or other vision changes, mental status changes or seizures, petechia, purpura beyond the site of vaccination, and/or easy bruising/bleeding. The medical management of thrombosis with thrombocytopenia is different from the management of isolated thromboembolic diseases. Investigators should follow available guidelines for the assessment and treatment of thrombotic thrombocytopenia (e.g., American Society of Hematology 2021British Society for Haematology 2021; CDC 2021). The use of heparin may be harmful and alternative treatments may be needed. Consultation with hematologists is strongly recommended".*	Because Vaxart's VXA-G1.1-NN investigational vaccine utilizes an adenovirus vectored design, and per the direction of the FDA, information on the risks of thromboembolism and /or thrombocytopenia have been added to Section 1.9 of the protocol.
1.9.2 Norovirus Challenge Risks	The amount of time expected to elicit acute watery diarrhea was changed to 12-72 hrs from 18 to 48 hrs	Information was updated based on the findings in the GI.1 norovirus titration study (VXA-NVV-201.1).
7.2 Visit 00: Screening Visit	Addition of •Blood Sample: coagulation tests, lab panel (PT/INR, aPTT and fibrinogen Subjects will be reminded to fast and refrain from ingesting solid food for at least 4 hours prior to the Day 1 study drug administration	A blood sample for coagulation testing was added at screening to evaluate for risk factors related to clotting. Based on findings from other Vaxart vaccine trials, it was determined that a 4 hour fast (rather than 8 hours) prior to study drug administration should be sufficient.
7.3 Visit 01 : Study Day 1, Study Drug Administration	Addition of: *Drug screen (urine and alcohol breath test)*	A drug screen was added to study day 1 (prior to vaccination) as part of the screening process.

7.5 Visit 03 : Study Day 28 [+30 days], 1 day prior to challenge	Addition of: *Any subject who is deemed ineligible will be discharged from unit prior to challenge. Subjects may return for re-evaluation of challenge eligibility within 30 days of their original Day 28 Visit. If they are assessed to be eligible for challenge at that time, they may be included for challenge with a subsequent cohort.*	A 30-day window was added to the pre-challenge visit (Day 28) to add flexibility for subjects that may not be eligible or available at the original visit timepoint. This change is not expected to impact the study immunogenicity and efficacy evaluations, however, will aid in reducing the screen failure rate for the challenge period.
7.11.2 Early Termination (Day 29 - Day 57; Challenge Phase	Addition of: *Subjects who did not undergo challenge and discontinue between Days 29 and Day 57 do not need the above listed ET assessments, but rather should be managed as early terminations during the Safety Follow-up Period (see Section 7.11.3)*	Information added for sake of clarification.
7.13 Immunogenicity Assessments	Addition of: *Additional exploratory immunogenicity assays may also be performed to further evaluate the activity of the VXA-G1.1-NN vaccine candidate on samples stored for future use (with subjects' consent). Note that not all sample timepoints may be relevant for the some of the analysis, so not all assays will be performed at all timepoints*	Information added for sake of clarification.
11.4 Final Analysis Plan	Deletion of: A preliminary report will be prepared by the SDCC after the primary clinical database is locked and all serological data through approximately 28 days post challenge are received. These analyses may be made available to the sponsor for planning subsequent trials and for publication. These analyses will not be used to make any decisions concerning the conduct of this trial.	Information was deleted as not relevant for the clinical protocol.
14.3 Informed Consent Process	Addition of: *The Informed Consent Form explains the storage and future use of specimens, and subjects will be asked to consent for future testing of samples*	Information added for sake of clarification.
Appendix E : List of Adverse Events of Special Interest	Addition of: *Coagulopathy* • *Acquired amegakaryocytic thrombocytopenia* • *Axillary vein thrombosis* • *Cerebral venous thrombosis* • *Disseminated intravascular coagulation* • *Hepatic vein thrombosis* • *Intracranial venous sinus thrombosis* • *Portal vein thrombosis*	The list of AESI to be monitored under this protocol was updated based on the most recent list of events received by FDA for Vaxart's oral Ad5 vaccine candidates.

	• *Pulmonary thrombosis* • *Severe fever with thrombocytopenia syndrome* • *Thrombocytopenia* • *Thrombotic thrombocytopenia purpura* • *Transverse sinus thrombosis* • *Vena cava thrombosis* • *Amegakaryocytic thrombocytopenia* • *Cavernous sinus thrombosis* • *Deep vein thrombosis* • *Embolism venous* • *Immune thrombocytopenia* • *Mesenteric vein thrombosis* • *Pulmonary embolism* • *Pulmonary venous thrombosis* • *Subclavian vein thrombosis* • *Thrombocytopenia purpura* • *Thrombosis* • *Vena cava embolism* • *Venous thrombosis*	

Additional minor wording, spelling and/or formatting modifications have been incorporated for added clarity and consistency.