Clinical Study Protocol

Protocol Title:	Evaluation of Infectivity and Illness of Norwalk GI.1 Virus Lot 001-09NV in the Human Challenge Model
Protocol Number:	VXA-G11-201.1
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Funding Sponsor:	Vaxart, Inc.
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Protocol Version Number: Amendment 1 Date:	1.1 21 September 2018

SIGNATURE PAGE/STATEMENT OF COMPLIANCE

Protocol Title: Evaluation of Infectivity and Illness of Norwalk GI.1 Virus Lot 001-09NV in the Human Challenge Model

Protocol Number: VXA-G11-201.1

Vaxart, Inc. _____ _____
David Taylor Date
Chief Medical Officer

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). 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/Independent Ethics Committee (IEC), except where necessary to eliminate an immediate hazard(s) to the trial subjects. 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: David Nguyen, MD

Site & Address: WCCT Global, Inc.

3545 Howard Way

Costa Mesa, CA 92626

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LIST OF ABBREVIATIONS AND ACRONYMS

AE	Adverse Events
ALT	Alanine aminotransferase
ASC	Antibody secreting cell
BT_{50}	histo-blood group binding antigen blocking antibody titer
BUN	blood urea nitrogen
CDC	Centers for Disease Control and Prevention
CFR	Code of Federal Regulations
CIOMS	Council for International Organizations of Medical Sciences
cGMP	Current Good Manufacturing Practices
CRF	Case Report Form
ELISA	Enzyme-linked Immunosorbent Assay
FDA	Food and Drug Administration
FUT2	gene encoding for α (1,2) fucosyltransferase
GI	Norovirus Genogroup I
GII	Norovirus Genogroup II
GC	Genome copies
GCP	Good Clinical Practices
GLP	Good Laboratory Practices
GMP	Good Manufacturing Practices
HBGA	Histo-blood group antigen
HBsAg	Hepatitis B surface antigen
HCV	Hepatitis C virus
HIPAA	Health Insurance Portability and Accountability Act
HIV	Human Immunodeficiency Virus
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
ISM	Independent Safety Monitor
NIAID	National Institute of Allergy and Infectious Diseases
NV	Norwalk Virus
NoV	Norovirus
NVG	Norovirus gastroenteritis
PBMC	Peripheral Blood Mononuclear Cells
PCR	Polymerase Chain Reaction
PHI	Protected Health Information
PI	Principal Investigator
QA	Quality Assurance
qRT-PCR	Quantitative Reverse Transcriptase Polymerase Chain Reaction
SAE	Serious Adverse Event/Serious Adverse Experience
SMV	Snow Mountain Virus, a Norovirus GII virus
SOP	Standard Operating Procedure
UNC	University of North Carolina at Chapel Hill
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

PROTOCOL SUMMARY

Study Title	Evaluation of Infectivity and Illness of Norwalk GI.1 Virus Lot 001-09NV in the Human Challenge Model
Protocol Number	VXA-G11-201.1
Sponsor	WCCT Global
IND Number	14697
Trial Phase	Phase 1
Investigational Sites	1-2 clinical sites with isolation wards
Investigational Products	Challenge strain: Norovirus G1.1 (Norwalk Virus Inoculum Lot 001-09NV)
Study Hypotheses	Norwalk virus (NV) will cause norovirus gastroenteritis (NVG) related to norovirus (NoV) infection in the challenge model in more than 50% of subjects challenged
Rationale	There is a need for safe, highly infectious NoV inocula for use in NoV vaccine-challenge studies to assess the efficacy of NoV vaccines and examine the immune response among vaccinated and unvaccinated subjects.

Study Objectives	Primary:
	• Evaluate the infectivity and safety of the human challenge model with NV inoculum as measured by the number infected, number that become ill, and the number of serious adverse events (SAEs).
	  • Infection status will be determined by: 1) virus detection in stool or emesis samples by quantitative reverse transcriptase-polymerase chain reaction (qRT-PCR) or 2) seroconversion through anti-NV antibody enzyme-linked immunosorbent assays (ELISAs).
	Secondary:
	• Determine the duration of viral shedding in stool and emesis samples after challenge.
	• Solicited gastrointestinal symptoms
	• Immunologic measurements of:
	  • Anti-NV Immunoglobulin G (IgG) and Immunoglobulin A (IgA)
	Exploratory:
	  • NV specific antibody response
	  • Histo-blood group binding antigen (HBGA) blocking antibody titers (BT_{50}).
	  • Antibody Secreting Cells (ASC) response

Study Endpoints	Primary Endpoints: • Occurrence of NVG within 7 days post-challenge (Study Day 9) in subjects for each dose group • Occurrence of SAEs in subjects for each dose group Secondary Endpoints: • Occurrence of any systemic solicited symptoms in subjects for each dose group (systemic solicited signs or symptoms are defined as diarrhea, vomiting, headache, nausea, fever, abdominal cramps or pain, abdominal gurgling or bloating, myalgia) • Severity of NVG using the modified Vesikari Scale up to 7 days post-challenge • Duration of NVG among challenged subjects up to 7 days post challenge • Occurrence of evidence of NV infection (with challenge strain) in subjects up to 28 days post-challenge

	• Percentage of subjects with seroconversion in serum anti-Norwalk pre-challenge to 28 days post-challenge • Time to cessation of gastro-intestinal illness • Duration of shedding Exploratory Endpoints: • Number (percent) of subjects with at least a 4-fold rise in NV-specific antibody titers post challenge. • Number (percent) of subjects that seroconvert post-challenge, subdivided by illness status. • Number (percent) of subjects with at least a 4-fold rise in HBGA BT_{50} from baseline compared to Day 28 • ASC IgA and IgG, Day 1 and Day 6 and/or Day 9 • Fecal IgA and saliva IgA, pre-challenge to post-challenge
Study Definitions	NVG is a composite endpoint for the analysis of clinical illness and is defined as meeting one or more definitions of NV Infection and one or more of the definitions of Acute Gastroenteritis. Acute Gastroenteritis: • Diarrhea: ≥ 3 loose or liquid stools or > 400 to 600 grams of loose or liquid stools produced in any 24-hour period or • Vomiting: ≥ 2 vomiting episodes in any 24-hour period or • One vomiting episode plus any loose or liquid stool in any 24-hour period or • One vomiting episode plus at least 2 of the following 5 events: headache, nausea, oral temperature ≥ 37.6°C, abdominal cramps or pains, abdominal gurgling or bloating or myalgia in any 24-hour period. NV Infection: • NV infection as detected by qRT-PCR in one or more post-challenge stool or emesis samples through Day 7 post-challenge (Study Day 9). • IgA/IgG ELISAs for anti-NV, ≥ 4-fold rise in titer in serum on Challenge Day 28 compared to pre-challenge.
Study Design	Once informed consent has been obtained from the subject and s/he is determined eligible for the study, the subject will be admitted to the isolation unit. Up to 16 secretor-positive, A or O blood type subjects will receive the NV inoculum. Eight subjects will receive the moderate challenge dose in the first challenge cohort, and 8 subjects will receive the higher dose in the second challenge cohort. Subjects will be monitored in the isolation unit for at least 5 days after challenge (+ 1 day) and will have additional follow-up at Days 9 ± 1, 14 ± 3, 28 ± 3 and 45 ± 3. During the inpatient stay and follow-up visits, emesis (if available), stool, blood, and saliva samples will be collected from study subjects for analysis of virus excretion and antibody response.

	Number of Subjects to be Challenged with Norwalk Virus Strain Lot 001-09 NV by Dose Number of subjects Lot 001-09 NV: Challenge 1: 8; Challenge 2: 8; Total: 8 Moderate dose, $3.6x10^5$ GC: Challenge 1: 8; Challenge 2: ; Total: 8 Higher dose, $1x10^6$ GC: Challenge 1: ; Challenge 2: 8; Total: 8 GC = genome copies
Study Population	Healthy adults 18 to 49 years old, inclusive. Subjects who are blood group A or O and H type-1 antigen secretor positive (by saliva test) will be selected for their susceptibility to NV.
Number of Subjects	Sixteen subjects will be enrolled in the study. A total of 8 subjects will receive the moderate dose and 8 will receive the higher dose. After an assessment of safety, 8 subjects will receive the higher dose in the second challenge.
Eligibility Criteria	Inclusion Criteria 1. Male or female between the ages of 18 – 49 years, inclusive 2. General good health, without significant medical illness, based on medical history, physical examination, vital signs, and clinical laboratories (CBC, chemistry, and urinalysis) as determined by the investigator in consultation with the research monitor and sponsor. 3. Willing to participate after written informed consent obtained. 4. Available for all planned visits and to spend at least 5 days in confinement. 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 30 at screening. 8. Female subjects must have a negative pregnancy test at screening and pre-challenge and fulfill one of the following criteria: a. At least 1 year post-menopausal; b. Surgically sterile; c. Use of oral, implantable, transdermal or injectable contraceptives for 60 days prior to challenge and until 60 days after challenge; i. A reliable form of contraception must be approved by the Investigator (e.g., double barrier method, Depo-Provera, intrauterine device, Norplant, oral contraceptives, contraceptive patches. ii. Male subjects must agree not to father a child or donate sperm from challenge until 60 days after challenge. 9. Available to return for follow-up visits following discharge from the inpatient unit and deliver stool specimens to the investigator promptly. Exclusion Criteria: 1. Presence of a significant medical condition, (e.g., 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 which in the opinion of the investigator precludes participation in the study. 2. History of cancer or cancer treatment within past 3 years (excluding basal cell carcinoma or squamous cell carcinoma) 3. Presence of immunosuppression or medical condition possibly associated with impaired immune responsiveness, including diabetes mellitus or angioedema. 4. Donation or use of blood or blood products within 4 weeks prior to challenge or planned donation during the study period 5. Diagnosed bleeding disorder or significant bruising or bleeding difficulties that could make blood draws problematic 6. Any condition that resulted in the absence or removal of the spleen 7. Evidence of confirmed infection with human immunodeficiency virus (HIV), hepatitis B surface antigen (HBsAg) or hepatitis C virus (HCV) with confirmatory assays. 8. Abnormal stool pattern (fewer than 3 per week or more than 3 per day). 9. Use of antibiotics, proton pump inhibitors, H2 blockers or antacids within 7 days before challenge and for 60 days after challenge. 10. Use of medication know to affect the immune function (e.g., systemic corticosteroids and others) within 7 days before challenge or for 60 days after challenge. 11. Regular use of nonsteroidal anti-inflammatory drugs, sulfonylureas, and angiotensin II blockers within 7 days before challenge and for 60 days after challenge. 12. Evidence of recent (within 2 months) or of current nonbacterial gastroenteritis suggestive of NV infection [vomiting or unformed or watery stools (> 2 during a 24-hour period)]. 13. Any gastroenteritis within the past 2 weeks. 14. Acute disease within 72 hours prior to challenge 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) 15. Stool sample with occult blood at screening 16. Positive stool/fecal for enteric pathogens (e.g., salmonella, campylobacter, E. coli 0157:H7, and shigella) as detected by the Biofire assay at screening. 17. Any significant hospitalization within the last year which in the opinion of the investigator or sponsor could interfere with study participation. 18. History of drug, alcohol or chemical abuse within 1 year prior to challenge 19. Positive urine drug screen for drugs of abuse or alcohol breath test at screening or baseline. 20. Consistent/habitual smoking within 2 months prior to challenge as per medical interview. Consistent/habitual smoking is defined as the smoking of

	one or more packs of cigarettes a day. Smoking will not be permitted during the inpatient stay. 21. Administration of any investigational vaccine, drug or device within 8 weeks preceding challenge, or planned use of the above stated for the duration of the study. 22. Other condition that in the clinical judgment of the investigator would jeopardize the safety or rights of a subject participating in the trial, would render the subject unable to comply with the protocol or would interfere with the evaluation of the challenge stage or the evaluation of the challenge stage. Occupational- 1. Living with or having daily contact with children age 5 years or less or a woman known to be pregnant or nursing. This includes significant contact at home, school, day-care, or equivalent facilities. 2. Living with or having daily contact with elderly persons aged ≥ 70 years or more, or infirmed, diapered individuals, persons with disabilities or incontinent persons. This includes work or visits to nursing homes and day-care or equivalent facilities. 3. Employment in the food service industry, such as restaurants, or cafeteria facilities. Specifically, this will include persons whose employment requires food handling and processing in the 4 weeks following challenge. 4. Health-care workers with patient contact expected in the 4 weeks following challenge. 5. Expected contact (through employment or at home) with immunocompromised persons (HIV-positive, receiving immunosuppressive medications such as oral steroids, anti-neoplastic agents) in the 4 weeks following challenge. 6. Employment as an airline flight attendant or cruise ship crew, scheduled to work in the 4 weeks following challenge. 7. Persons planning to be in a confined environment (e.g., a cruise, camp etc.) in the 4 weeks following challenge.
Study Duration	• Sixty days pre-screening, recruitment and study specific screening • Five days in isolation ward (minimum) • Four follow up clinic visits after discharge on Days 9, 14, 28, and 45 (Day 45 is a phone call)
Randomization	This is an open label study. The challenge cohorts will be enrolled sequentially.
Microbiological Assessments	During the challenge phase all stools will be weighed/measured for volume and graded. All stools will be collected daily and tested for NoV.
Safety Assessments/ Clinical Monitoring	Challenge period and safety follow up: • Unsolicited adverse events (AEs) through Day 45 • Pre-challenge and daily throughout the confinement period post-challenge: Following challenge, study subjects will be monitored for solicited gastrointestinal and other symptoms (systemic solicited signs or symptoms are defined as diarrhea, vomiting, headache, nausea, fever, abdominal cramps or pain, abdominal gurgling or bloating, myalgia) and vital signs in the clinical

	research unit every 2 hours while awake during the first 48 hours post-challenge, and then every 8 hours on Days 3 to 5 post-challenge. ○ Assessment of safety laboratory panels (chemistry and hematology) on the day of discharge. Additional time points may be collected if clinically indicated per the discretion of the Investigator. • Follow-up clinic evaluations on Days 9, 14, 28, and 45 (Day 45 is a phone call)
Sample Size Estimate/Data Analysis	The purpose of this study is to verify that Lot 001-09 NV is infectious and able to cause illness and to determine the titer of NV inoculum which causes infectivity in >50% of subjects. A higher and moderate inoculum dose will be studied. The dose that is both safe and able to induce illness in the majority of subjects will be chosen for use in vaccine evaluation studies.

SCHEMATIC OF STUDY DESIGN

A total of 16 eligible healthy young adults will be challenged with either the moderate or higher dose of NV Lot 001-09 NV. Once informed consent has been obtained from the subject and s/he is determined eligible for the study, the subject will be admitted to the clinical research unit to receive the NV inoculum. Subjects will be monitored in isolation for at least 5 days (+ 1 day) after challenge. During the inpatient stay, emesis (if available), stool, blood, and saliva samples will be collected from study subjects. Discharge from the research unit will be contingent upon: the absence of diarrhea and absence of moderate or high-grade objective reactogenicity (diarrhea, fever, and vomiting). After discharge subjects will have additional follow-up at Days 9 ± 1, 14 ± 3, 28 ± 3, and 45 ± 3. The study subjects will be divided into 2 groups (**Table 1**). In challenge 1, 8 subjects will receive the moderate dose. In challenge 2, 8 subjects will receive the higher dose.

Table 1: Number of subjects to be challenged according to dose

	Number of subjects		
Lot 001-09 NV	Challenge 1	Challenge 2	Total
Moderate dose, $3.6x10^5$ GC	8		8
Higher dose, $1x10^6$ GC		8	8

GC = genome copies

INTRODUCTION: BACKGROUND INFORMATION AND SCIENTIFIC RATIONALE

1.1 Norovirus disease etiology, transmission and epidemiology

Noroviruses (NoV) are the major cause of epidemic gastroenteritis in the United States and a significant cause of severe diarrhea in young children in developing countries (1, 2). NoVs are also the most frequent cause of acute gastroenteritis after ingestion of raw shellfish (3-5). NoV infection causes symptoms of vomiting, watery diarrhea, nausea, abdominal cramps, fever, and general malaise. NoVs are classified by the Centers for Disease Control and Prevention (CDC) and National Institute of Allergy and Infectious Diseases (NIAID) as Bioterrorism Category B Priority Pathogens based on their high transmissibility, low infectious dose, and serious public health and economic impact. Transmission of NoV occurs via ingestion of fecal-contaminated food and water, exposure to contaminated fomites or aerosolized emesis, and direct person-to-person contact (6-14). In rare cases, transmission can occur through organ transplantation (13, 14). A low median infectious dose of 18 genomic copies has been described (15). In certain individuals, the virus can be shed for 3 weeks or longer after infection (16-18). These viruses are the second most important cause of acute gastroenteritis in young children (19, 20) and may cause about 20% of endemic gastroenteritis in families (21). Each year in the United States, the public health impact of NoV is evidenced by the estimated 23 million infections that result in an estimated 50,000 hospitalizations and 310 fatal cases (22). Gastroenteritis induced by NoV is self-limiting and rarely fatal, especially in healthy adults. Fatality in children and the elderly is usually caused by severe dehydration after NoV infection (2, 23, 24). No vaccine is currently available.

NoVs belong to the family Caliciviridae and can be subdivided into 5 distinct genogroups. Genogoups I, II, and IV cause disease in humans, and genogroups I and II are the major cause of infection and outbreaks in humans. Each genogroup is further subdivided into clusters, and clusters are divided into strains. There are at least 8 clusters for genogroup I (GI) and 17 clusters for genogroup II (GII) (25). The first NoV to be identified is Norwalk virus (NV) and is a prototype virus for the G I.1 cluster.

Several genetic determinants of host susceptibility to NoV infection have been observed. Parrino et al. reported that a subset of subjects was repeatedly susceptible to NV infection and a second subset was repeatedly resistant to infection after challenged with NV (26). Our group found that a member of the ABO histo-blood group family, the FUT2 allele (ie, the gene encoding for α (1,2) fucosyltransferase), conferred susceptibility to NV infection (27). Individuals who were homozygous recessive for the FUT2 gene are considered secretor negative (28) and do not become infected regardless of NV dose. In addition, saliva samples from these individuals did not bind to NV virus-like particles (VLPs). The FUT2 gene encodes an α (1,2) fucosyltransferase that produces the carbohydrate H type 1 found on epithelial cells and in mucosal secretions (29). In addition, individuals of the B blood group are more resistant to NoV infection, while those of the O blood group are more susceptible to NoV infection (27, 30). Together, this data suggests that the ABO histo-blood group components, including FUT2, may be necessary for NV binding and infection and are, therefore, important genetic determinants of susceptibility to NV infection. These genetic components apply only to NV. Other NoV strains may infect individuals who are "genetically resistant" to 1 strain of NoV (31). For example, we have shown that infection with Snow Mountain virus (SMV) (GII.2) is not secretor dependent (32).

NoV strains are genetically diverse and infection with a single strain does not confer long-term sterilizing immunity, but rather short-term protection (33). Similarly, long-term immunity has been difficult to achieve for some enteric vaccines, possibly due to the rapid decline of intestinal immunoglobulin A (IgA) compared with longer-term serum Immunoglobulin G (IgG) responses (34). Mucosal IgA likely plays a pivotal role in NoV protection, but human challenge studies have shown that serum IgA, memory B cell responses, and serum histo-blood group antigen (HBGA) blocking titers (BT_{50}) are all potential immunological correlates of protection (35-39).

1.2 Previous experience with the Norwalk GI.1 virus

In a study by Atmar et al. subjects received a challenge dose of 8fIIa in a multisite trial to evaluate a NV vaccine (35). 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) (36). 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 2**). 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 2: Norwalk virus infection and illness rates after challenge with NV 8fIIa.

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. (35)		

After the Atmar study, NV strain 8fIIa was passed in a human and the resultant strain was designated 8fIIb. Teunis et al. compared the infection and illness rates for 8fIIa and 8fIIb (15). 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 1** 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 (40).

Teunis et al. exposed 53 subjects to doses of 8fIIa ranging from $3x10^1$ to $3x10^8$ GCs (15). A dose response was observed with a peak illness rate in 6/8 (75%) of subjects at a dose of $3x10^5$ GC (**Table 3**). 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 3: Dose response for Norwalk strain 8fIIa and 8fIIb

	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					
7×10^5	8	3	37.5	2	25.0
7×10^6	18	14	77.8	7	38.9
2×10^7	1	1	100.0	NA	
Total	27	18	66.7	9	33.3

Source: adapted from Teunis (15)

NV strain 8fIIb was used as a human challenge strain in 2 other studies (**Table 4**) 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 (41, 42).

Table 4: Summary of Norovirus Challenge Studies Completed in C. Moe's Lab (Emory Univ.)

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. (43) among 13 infected subjects NV 8fIIb were low grade fever, nausea, vomiting and diarrhea (**Table 5**).

Table 5: Symptoms reported after experimental 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)

1.3 Study Rationale

There is a need for safe, highly infectious NoV inocula for use in NoV vaccine-challenge studies to assess the efficacy of NoV vaccines and examine the immune response among vaccinated and unvaccinated subjects.

Vaxart hypothesizes that NV infection and illness can be elicited following NV challenge. The NV Lot 001-09 NV inoculum will be tested for safety and dose as an investigational challenge product in this study. Assessing the safety of the NV inoculum, the immune determinants of host susceptibility to NV, and the immune response to NV challenge and infection will support or reject this hypothesis.

Selection of study population. This study will enroll normal healthy subjects, male or female, between the ages of 18 and 49 who fulfill all eligibility criteria. Because illness is being induced in subjects, it is important to make sure that the subjects have no preexisting conditions that may be complicated by NVG. Children (younger than 18) will be excluded because they may not fully understand and comply with study procedures, are often in contact with large groups of other susceptible children (schools, daycare, etc.), and are not the target population for the NoV vaccines currently under development. Older adults will also be excluded because of a possible weaker immune system, a higher likelihood of preexisting conditions and complications from NoV infection, and possible contact with large groups of other susceptible older adults (e.g., hospitals, elderly care facilities).

Route of administration. The study subjects will be challenged with an oral dose of safety-tested NV inoculum in water. This inoculum will be preceded and followed by an oral dose of bicarbonate (baking soda) dissolved in water. Bicarbonate will neutralize stomach acidity for NV infection. Bicarbonate has been used in all human NoV challenge studies to neutralize stomach acid. For each subject, the 1 or 3 vials of inoculum (1 or 3 mL) will be diluted in 100 mL of sterile distilled water immediately prior to challenge because it makes it easier for the subjects to ingest the inoculum. The oral route is easy to administer and there is high compliance among all our subjects in our previous studies and the studies of others.

NV strain Lot 001-09NV has not been administered to human subjects, however, as noted above, there is extensive experience with the parent strains NV 8fIIa and 8fIIb. To assess the safety and basic infectivity of the NV inoculum, the first 8 subjects will be challenged with a moderate dose of NV ($1x10^5$ GC, approximately 0.5 mL of NV inoculum [1 mL vial of inoculum]) before testing a higher dose.

The second challenge will be with 8 subjects at a higher dose. Based on experience with previous NoV challenge studies and the NV dose-response model, it is proposed to challenge these 8 subjects with dose of 10^6 GC of NV (3 vials of inoculum per subject). In the previous NV challenge studies, infection rates ranging from 37.5% to 87.5% were observed among secretor-positive status subjects who ingested NV doses between $3.24x10^5$ and $6.92x10^6$ GC (47, 26). An increase in illness rates was not observed with doses greater than 10^6 NV GC in the previous challenge studies. A dose of 10^6 GC for NV should induce an infection rate of 55 to 79% as determined by the dose-response model.

1.4 Potential Risks and Benefits

There are no benefits to the subjects for their participation in this research study. Subjects who undergo challenge with NoV should expect to have diarrhea; in some cases the diarrhea can be severe. NoV illness may also be associated with nausea, vomiting, abdominal cramping or discomfort, loss of appetite, muscle aches, and tiredness. The electrolyte losses with the secretory diarrhea may result in cardiac conduction abnormalities, if uncorrected. Intravascular volume losses with diarrhea can be associated with postural hypotension, shock, and death, if uncorrected.

1.4.1 Known Potential Risks

1.4.1.1 Challenge with NV Lot 001-09NV

The intended NV dose of $3.6x10^5$ or $1x10^6$ GC is expected to elicit acute watery diarrhea within 18-48 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) 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 (i.e., hand washing with soap and water after defecation) are unlikely to transmit the strain person to person.

1.4.1.2 Risk of Allergic Reaction

There is a very rare risk for an allergic reaction to the challenge inoculum. Allergic reactions generally can range from mild (for example, skin rash, itching, swelling, or numbness) to severe (for example, low blood pressure, difficulty breathing, shock, heart arrest, or death). The symptoms of a mild allergic reaction typically go away without treatment, but some cases may require treatment with antihistamines or steroids (medications used to treat allergic reactions). In the case of more severe allergic reactions such as shock, immediate and intensive medical treatment is necessary. Although the risk of an allergic reaction is very small, epinephrine, antihistamines, and other equipment will be available to treat anaphylaxis or immediate-type hypersensitivity reactions.

1.4.1.3 Risk with Pregnancy

NoV infection in a pregnant woman can harm the fetus. All female study subjects will be tested for pregnancy prior to challenge. Any pregnancies which occur between challenge and Day 28 post-challenge will be reported and followed up.

1.4.1.4 Risk with Blood Draws

Blood will be drawn at several times during the study and may also be performed to help manage the diarrheal illness. The drawing of blood may cause pain, bruising, feeling faint, fainting, needle site infections, swelling, and rarely other infections. Bruising at the site of blood drawing can be prevented by applying pressure for several minutes. To reduce the risk of infection, the skin site is prepared with an alcohol wipe and sterile equipment is used.

1.4.1.5 Risks to Confidentiality

Personal health information will be collected as a part of this study and efforts will be made to maintain confidentiality. There is a small risk of loss of confidentiality that an unauthorized person may gain access to viewing the research records. To maintain confidentiality, all study records will be stored in a secure location, such as a locked office and/or locked cabinet. Electronic data will be password-protected. Study records and specimens obtained will be coded. Research records will only be shared with authorized personnel and only in connection with carrying out the obligations relating to the study. Every effort will be made to keep the records as confidential as possible, within the limits of the law.

1.4.1.6 Known Potential Benefits

This is a healthy subject study which does not provide any guarantee of benefit. The benefit is largely the scientific knowledge to be gained from the study.

1.5 Overall Development Strategy

The purpose of this study is to evaluate the infectivity and illness rate of two different doses of the NV inocula in a human challenge model as measured by the number infected, number that become ill, and the number of serious adverse events (SAEs). Depending on the results this model may be used to evaluate vaccine efficacy of the Vaxart NoV vaccine.

2 HYPOTHESIS, OBJECTIVES AND PURPOSE

2.1 Study Hypothesis / Hypotheses

Norwalk norovirus G1.1 Lot 001-09NV at a moderate or high dose will cause NoV gastroenteritis (NVG) in the challenge model in ≥ 50% of subjects challenged.

2.2 Study Objectives

2.2.1 Primary Objective

- Evaluate the infectivity and safety of the human challenge model with NV inoculum as measured by the number infected, number that become ill, and the number of SAEs.
    - Infection status will be determined by: 1) virus detection in stool or emesis samples by quantitative reverse transcriptase-polymerase chain reaction (qRT-PCR) or 2) seroconversion through anti-NV antibody enzyme-linked immunosorbent assays (ELISAs).

2.2.2 Secondary Objectives

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

2.2.3 Exploratory Objectives:

- NV specific antibody response
- HGBA BT_{50}.

- Antibody Secreting Cells (ASC) response

3 STUDY DESIGN AND ENDPOINTS

3.1 Description of the Study Design

A total of 16 eligible healthy young adults will be challenged with either the moderate or higher dose of NV strain Lot 001-09NV. Once informed consent has been obtained from the subject and s/he is determined eligible for the study, the subject will be admitted to the clinical research unit to receive the NV inoculum. Subjects will be monitored in isolation for at least 5 days (+ 1 day) after challenge. During the inpatient stay, emesis (if available), stool, blood, and saliva samples will be collected from study subjects. Discharge from the research unit will be contingent upon: the absence of diarrhea and absence of moderate or high-grade objective reactogenicity (diarrhea, fever, and vomiting; see Appendix D). After discharge subjects will have additional follow-up at days 9 ± 1, 14 ± 3, 28 ± 3, and 45 ± 3. The study subjects will be divided into 2 groups (**Table 6**). In Challenge 1, 8 subjects will receive the moderate dose and subsequently in Challenge 2, 8 subjects will receive the higher dose.

Table 6: Number of subjects to be challenged according to dose and study site

	Number of subjects		
Lot 001-09 NV	Challenge 1	Challenge 2	Total
Moderate dose, $3.6x10^5$ GC	8		8
Higher dose, $1x10^6$ GC		8	8
GC = genome copies			

3.2 Study Endpoints

3.2.1 Primary Endpoints

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

3.2.2 Secondary Endpoints

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

3.2.3 Exploratory Endpoints

- Number (percent) of subjects with at least a 4-fold rise in NV-specific antibody titers post challenge.
- Number (percent) of subjects that seroconvert post-challenge, subdivided by illness status.

- Number (percent) of subjects with at least a 4-fold rise in HBGA BT50 from baseline compared to Day 28
- ASC IgA and IgG, Day 1 and Day 6 and/or Day 9
- Fecal IgA and saliva IgA, pre-challenge to post-challenge

4 STUDY ENROLLMENT AND WITHDRAWAL

4.1 Subject Inclusion Criteria

1. Male or female between the ages of 18-49 years, inclusive
2. General good health, without significant medical illness, based on medical history, physical examination, vital signs, and clinical laboratories (CBC, chemistry, and urinalysis) as determined by the investigator in consultation with the research monitor and sponsor.
3. Willing to participate after written informed consent obtained.
4. Available for all planned visits and to spend at least 5 days in confinement.
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 30 at screening.
8. Female subjects must have a negative pregnancy test at screening and pre-challenge and fulfill one of the following criteria:
    a. At least 1 year post-menopausal;
    b. Surgically sterile;
    c. Use of oral, implantable, transdermal or injectable contraceptives for 60 days prior to challenge and until 60 days after challenge;
        - A reliable form of contraception must be approved by the Investigator (e.g., double barrier method, Depo-Provera, intrauterine device, Norplant, oral contraceptives, contraceptive patches.
        - Male subjects must agree not to father a child or donate sperm from challenge until 60 days after challenge.
9. Available to return for follow-up visits following discharge from the inpatient unit and deliver stool specimens to the investigator promptly.

4.2 Subject Exclusion Criteria

1. Presence of a significant medical condition, (e.g., 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 which in the opinion of the investigator precludes participation in the study.
2. History of cancer or cancer treatment within past 3 years (excluding basal cell carcinoma or squamous cell carcinoma)
3. Presence of immunosuppression or medical condition possibly associated with impaired immune responsiveness, including diabetes mellitus or angioedema.
4. Donation or use of blood or blood products within 4 weeks prior to challenge or planned donation during the study period
5. Diagnosed bleeding disorder or significant bruising or bleeding difficulties that could make blood draws problematic
6. Any condition that resulted in the absence or removal of the spleen
7. Evidence of confirmed infection with human immunodeficiency virus (HIV), hepatitis B surface antigen (HBsAg) or hepatitis C virus (HCV) with confirmatory assays.
8. Abnormal stool pattern (fewer than 3 bowel movements per week or more than 3 per day).

9. Use of antibiotics, proton pump inhibitors, H2 blockers or antacids within 7 days before challenge and for 60 days after challenge.
10. Use of medication know to affect the immune function (e.g., systemic corticosteroids and others) within 7 days before challenge or for 60 days after challenge.
11. Regular use of nonsteroidal anti-inflammatory drugs, sulfonylureas, and angiotensin II blockers within 7 days before challenge and for 60 days after challenge.
12. Evidence of recent (within 2 months) or of current nonbacterial gastroenteritis suggestive of NV infection [vomiting or unformed or watery stools (> 2 during a 24-hour period)].
13. Any gastroenteritis within the past 2 weeks.
14. Acute disease within 72 hours prior to challenge 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)
15. Stool sample with occult blood at screening
16. Positive stool/fecal for enteric pathogens (e.g., salmonella, campylobacter, E. coli 0157:H7, and shigella) as detected by the Biofire assay at screening.
17. Any significant hospitalization within the last year which in the opinion of the investigator or sponsor could interfere with study participation.
18. History of drug, alcohol or chemical abuse within 1 year prior to challenge
19. Positive urine drug screen for drugs of abuse or alcohol breath test at screening, or baseline.
20. Consistent/habitual smoking within 2 months prior to challenge. Consistent/habitual smoking is defined as the smoking of one or more packs of cigarettes a day. Smoking will not be permitted during the inpatient stay.
21. Administration of any investigational vaccine, drug or device within 8 weeks preceding challenge, or planned use of the above stated for the duration of the study.
22. Other condition that in the clinical judgment of the investigator would jeopardize the safety or rights of a subject participating in the trial, would render the subject unable to comply with the protocol or would interfere with the evaluation of the challenge stage or the evaluation of the challenge stage.

Occupational:

1. Living with or having daily contact with children age 5 years or less or a woman known to be pregnant or nursing. This includes significant contact at home, school, day-care, or equivalent facilities.
2. Living with or having daily contact with elderly persons aged 70 years or more, or infirmed, diapered individuals, persons with disabilities or incontinent persons. This includes work or visits to nursing homes and day-care or equivalent facilities.
3. Employment in the food service industry, such as restaurants, or cafeteria facilities. Specifically, this will include persons whose employment requires food handling and processing in the 4 weeks following challenge.
4. Health-care workers with patient contact expected in the 4 weeks following challenge.
5. Expected contact (through employment or at home) with immunocompromised persons (HIV-positive, receiving immunosuppressive medications such as oral steroids, anti-neoplastic agents) in the 4 weeks following challenge.

6. Employment as an airline flight attendant or cruise ship crew, scheduled to work in the 4 weeks following challenge.
7. Persons planning to live in a confined environment (e.g., a cruise, camp, etc.) in the 4 weeks following viral challenge.

4.3 Withdrawal from 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

Every effort will be made to undertake protocol-specified safety follow-up procedures to capture AEs, SAEs. In the event of withdrawal from study, reasonable efforts should be made to conduct the following procedures:

- Review diary card/ memory aid if still in use prior to withdrawal
- Updating any ongoing AE/SAEs that remain ongoing at time of subject's last visit prior to withdrawal
- Query about AEs, SAEs and concomitant medications if the interval between the subject's last visit and the time of withdrawal is within the protocol defined reporting period
- Physical examination
- Blood for safety laboratory testing if withdrawal occurs before visit 28
- Update contact information

4.4 Premature Termination or Suspension of Study

This study may be suspended or prematurely terminated if there is sufficient reasonable cause. Written notification, documenting the reason for study suspension or termination, will be provided by the suspending or terminating party to investigator, funding agency, the IND/IDE sponsor and regulatory authorities. If the study is prematurely terminated or suspended, the PI will promptly inform the Instituitional Review Board (IRB) and will provide the reason(s) for the termination or suspension.

Circumstances that may warrant termination or suspension include, but are not limited to:

- Determination of unexpected, significant, or unacceptable risk to subjects
- Insufficient compliance to protocol requirements
- Data that are not sufficiently complete and/or evaluable
- Determination of futility

Study may resume once concerns about safety, protocol compliance, data quality is addressed and satisfy the sponsor, IRB and/or Food and Drug Administration (FDA).

5 STUDY AGENT

5.1 Study Agent(s) and Control Description

The inoculum for NV 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 the University of North Carolina at Chapel Hill (UNC). From there, the inoculum was sent to an outside contractor for safety testing. The preparation, qualification, and quantification of this inoculum was submitted to the FDA for investigational new drug application (IND) review.

5.2 Product Description

5.2.1 Acquisition

The clinical grade product will be obtained from the NoV Processing Facility located at the University of North Carolina at Chapel Hill. The facility is designed to produce inocula of NoV under Current Good Manufacturing Practices (cGMP). Briefly, the inoculum used in the present study was prepared as follows. Stool samples were obtained from NV-infected donors who have passed a health screening. NV was extracted from the virus-containing stool sample by a series of 5-6 washes with sterile water. Washes were performed by manually creating a 20% stool suspension in water followed by high speed centrifugation to pellet solid material. The supernate was removed to a clean tube and a volume of fresh sterile water was added back to the stool sample. The stool sample was then resuspended, mixed, washed, and centrifuged again to pellet solids. This process was then repeated until all washes were completed. Pooled washes created a partially-purified bulk substance. Washes from different donor samples were not included in this pool. Samples from the partially purified bulk material were then removed for specific lot release tests including viral titer of the pooled material.

The total volume of the material was then filtered twice through 0.2 micro filters and again pooled as bulk purified product. Samples from the purified bulk material were then removed for specific lot release tests and viral titer tests. The material was stored at 4ºC until the results of the pooled viral titer were known. The material was then portioned out and diluted with sterile water to the desired concentration(s) for filling bulk containers for different sublots and/or the single use vials required for the specific clinical study. The material was then filled and frozen down at -80ºC until the completion of all lot release tests, the final qualification and release of the material, and the shipment to the clinical site.

Prior to use in patients, the product, acquired as a single-patient/single-use vial, undergoes final processing as described in Appendix B.

5.3 Formulation, Appearance, Packaging, and Labeling

5.3.1 Formulation

Both the filled, purified product and the final patient use material are formulated in pure, distilled water with no other additives. Both the filled, purified product and the final subject use material may be formulated in a range of different concentrations of virus to allow for the creation of further sub-lots of material at new concentrations of virus, and/or changes in dosing regimens, respectively.

5.3.2 Packaging

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 final patient use material is packaged in sterile cups.

5.3.3 Labeling

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.

5.4 Product Storage and Stability

5.4.1 Storage

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.

5.4.2 Stability

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.

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

5.5.1 Investigational agent: Norwalk virus Lot 001-09NV

The agent used in this subject challenge trial is a safety-tested NV inoculum (Lot 001-09NV, IND 14697).

5.5.2 Route of administration

Subjects will receive a single oral dose of safety-tested inocula. The oral route was chosen because of ease of administration and successful precedent with this route from all other NoV challenge studies.

5.5.3 Norovirus dosage

Subjects will be challenged with either $3.6x10^5$ or $1x10^6$ GC of NV based on information from past challenge studies and the NV dose-response model. The dose-response model and experience with this dose indicates that this dose is sufficient to result in an infection rate between 55% to 79% among susceptible, secretor-positive status individuals. The moderate dose is a single-patient dose (one 1-mL vial for a total of 1 mL of NV inoculum Lot 001-09NV, IND 14697) and the high dose is a single-patient dose (three 1-mL vials for a total of 3 mL of NV inoculum Lot 001-09NV, IND 14697).

5.5.4 Frequency of administration

The intervention will be a single oral challenge with NV inoculum.

5.5.5 Preparation of dose

For the moderate dose one (1) vial and for the high dose three (3) vials per subject will be thawed under sterile conditions and swabbed with disinfectant. One or three vials 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. The sealed cup will be transported on ice to the subject for challenge. From previous work, the inoculum in water will be stable and infectious for at least 61 days.

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 inocula spills the inocula, the affected area must be immediately cleaned with a 10% bleach solution. Hands should be washed thoroughly with soap and water.

5.5.6 Starting Dose and Dose Escalation Schedule

The starting dose of Lot 001-09NV, 1 vial containing approximately $3.6x10^5$ GC, will be administered orally to eight subjects. If this dose is well tolerated, a dose of 3 vials containing approximately $1x10^6$ GC will be administered orally to the second cohort of 8 subjects contingent upon review of safety and infectivity data from cohort 1.

5.5.7 Dose Adjustments/Modifications/Delays

Not applicable

5.5.8 Product Storage and Stability

The storage and final preparation of subject doses will be performed at the study site pharmacy. In general, the overall process for shipping virus from the manufacturing facility in North Carolina, storing it in the research site pharmacy, and performing the final manipulations are as follows. Once the virus has completed all lot release testing and a certificate of analysis has been signed off on, the inocula are shipped frozen by overnight courier to the study sites. Data loggers and shipping documents are included in the shipping container to monitor temperature and document the transfer of the materials. When the virus is received at the study site, the materials and the container are inspected, and the status of the product is recorded. The material is then placed in a controlled -80˚C freezer and its location in the freezer is recorded in the freezer records. Clinical material is stored in a dedicated compartment of the freezer and is separated from any other materials in the freezer.

To prepare subject doses, the appropriate amount of material for the dose is removed from the freezer and its removal is recorded in the freezer records. The material is transferred to a biosafety cabinet in the laboratory and allowed to thaw at room temperature. The Biosafety cabinet is broken down and cleaned/sanitized prior to use and it is dedicated for use only with the product being worked on throughout the final preparation process. The inoculum vials (1 mL each) will be thawed under sterile conditions and swabbed with disinfectant. The inocula (1 mL or 3 mL) 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 sterile cup must be sealed and transported on ice to the subject for challenge. From previous work, the inocula in water will be stable and infectious for at least 61 days.

5.6 Study Agent Accountability Procedures

The site pharmacist is required to maintain complete records of all study products received from the Sponsor (or designee) and will be responsible for maintaining an accurate record of subject dosing, and an accountability record of the viral inocula for this study. The site pharmacist will also be responsible for ensuring the security of these documents. Partially used vials will 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 study products.

6 STUDY PROCEDURES AND SCHEDULE

See Appendix C for The Schedule of Study Visits and Evaluations.

6.1 Study Screening

6.1.1 Visit 00A. Pre-Screening visit (Outpatient; Days -60 to Screening)

The purpose of the first clinical visit is to pre-identify potential voluneers for secretor status and ABO blood type. Since 80% of a population is secretor positive and secretor positivity is an eligibility criterion, we need to screen over 20 eligible individuals to reach our target population of 16 secretor-positive subjects. However, we expect to screen more than 50 secretor-positive individuals to identify 16 that meet all the study inclusion and exclusion criteria. Healthy men and women 18 to 49 years of age will be recruited from the areas surrounding the study site(s) in the USA).

Pre-screening visit checklist

- Informed Consent
- ABO blood typing
- Saliva test for secretor status
- Demographics
- Eligibility criteria

Visit 00A pre- screening tests will be conducted under a separate pre-screening ICF. Eligible subjects will be required to sign a separate Informed Consent Forms (ICF) for study participation at the screening visit. If the subject is secretor negative or blood type B or AB (genetically resistant), the subject will be told he or she does not fulfill eligibility criteria and will not be enrolled in the study.

6.1.2 Visit 00B. Screening visit (Outpatient, Days -30 to -3)

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 designated 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 (pregnancy will be assayed by blood test). 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 to come to the inpatient unit for a 5-day admission. Subjects will be asked to bring a screening stool sample before they receive the NV challenge. The screening stool specimen will be tested for enteric pathogens and occult blood.

Screening visit checklist

- Demographics
- Medical interview
- Eligibility criteria
- Physical Examination
- Vital signs
- Concomitant medications
- ECG
- Blood screen for HIV, HBsAg and HCV

- Blood for safety laboratory evaluation
- Urinalysis
- Drug Screen (Urine, alcohol breath test)
- Stool sample for enteric pathogens and occult blood
- Serum Pregnancy test (females)

6.2 Inpatient Containment Period

6.2.1 Visit 00C. Challenge Baseline - Acclimatization (Day 1, 1 day prior to challenge)

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 on the inpatient unit. Subjects will also be monitored for evidence that a subject may demonstrate behaviors which might pose a safety risk to themselves, other subjects, or staff 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, prior to challenge.

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

The following assessments will be made as described in Appendix C:

- Medical Interview
- Targeted physical exam
- Vital signs
- Blood sample for safety laboratory tests
- Urinalysis
- Drug Screen (Urine, alcohol breath test)
- Urine pregnancy test (females)
- Blood samples for immunology
- Fecal sample for immunology
- Saliva sample for immunology

6.2.2 Visit 01. Challenge Day (Day 2)

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.

Subjects will have fasted overnight and 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, IND 14697) suspended in 100 mL of distilled water.

6.2.3 Visits 01 – 05. Post-Challenge Observation Period (Day 2 through Day 6 [+1 Day)

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

- Study subjects will be monitored for gastrointestinal symptoms and vital signs in the clinical research unit every 2 hours while awake during the first 48 hours post-challenge, and then every 8 hours on Day 3 to challenge phase discharge.
- Fecal samples will be collected.

- All stools will be graded for consistency (grade 1 to 5) and any diarrheal stool (grade 3 or higher) will be weighed.
- Saliva samples will be collected
- All emesis will be weighed and an emesis sample collected.
- Following challenge, symptoms will be assessed and the maximum symptom severity for the day will be determined using a standard rating scale.
- Anticipated subjective symptoms include: diarrhea, vomiting, headache, nausea, fever, abdominal cramps or pain, abdominal gurgling or bloating, myalgia. These adverse symptoms will be graded.
- The maximum temperature, total diarrheal stool volume, number of diarrheal stools, total vomitus volume, and number of vomiting episodes will be calculated for each 24 h period during the inpatient stay.
- On day of discharge (Day 6 + 1 day)
    - Blood sample will be collected for safety labs and PBMC isolation
    - Diary card will be distributed
    - Discharge criteria must be assessed and met (per checklist)

The nursing staff will monitor the subject's vital signs and assess for signs and symptoms of acute NoV infection every 2 hours while awake and while the subject is in the isolation unit over the first 48 hours. Rating scales (Appendix D and E) 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 will also be collected during the inpatient stay. During the first 5 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 5 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 (See analysis section 10.3 and Appendix C, Schedule of Events). 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 (see Appendix C, Schedule of Events).

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

6.2.3.1 Discharge Criteria

Study subjects will be evaluated for discharge 5 days after challenge (Day 6). If subject is still symptomatic with NV illness and it is clinically indicated, they will remain in the isolation unit for an additional day (Day 7). 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 9 ± 1 (first follow-up visit). Subjects will also be given a memory aid (diary) to chart any change in signs, symptoms, or medication use following discharge. This memory aid will be returned to the clinical site at the day 9 visit.

6.2.3.2 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 ensure that 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.

6.3 Outpatient Visits

6.3.1 Visit 06. Outpatient Clinic Visit 1 (Day 9 ± 1)

Symptom memory aids will be collected. A medical interview, targeted physical exam if indicated and vital signs will be performed. Whole blood specimens will be collected and processed for PBMC isolation. Saliva specimens will be collected. Subjects will bring in a stool collected within 24 hours of day 9 visit. Subjects will be given stool collection kits to take home, so they can bring a stool specimen collected within 24 hours of each of their follow-up visits. 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 clinic, stool samples will be processed per Lab Manual.

6.3.2 Visit 07. Outpatient Clinic Visit 2 (Day 14 ± 3)

Subjects will undergo the following evaluations:

- Medical Interview
- Targeted physical exam will be performed if indicated.
- Vital signs (temperature, blood pressure, heart rate, breathing rate)
- Stool specimen to assess viral shedding.

6.3.3 Visit 08. Outpatient Clinic Visit 3 (Day 28 ± 3)

Subjects will undergo the following evaluations:

- Medical Interview
- Targeted physical exam will be performed if indicated.
- Vital signs (temperature, blood pressure, heart rate, breathing rate)
- Urine pregnancy test (females)
- Blood specimens for immunology tests
    - 20 ml for serum
- Stool specimen to assess viral shedding.

6.3.4 Visit 09. Final Follow-up phone call (Day 45 ± 3)

A final follow up visit can be completed by phone. The visit will only be for the reporting of SAEs.

6.4 Unscheduled Visit(s)

Subjects whose Day 28 stool sample is positive for norovirus shedding will be asked to return to clinic for additionalfecal testing on or before Day 45. Any need for further follow up will be evaluated on a case by case basis. Subjects that experience any serious or severe AEs or experience an event of concern can be scheduled for an outpatient visit to have further evaluation. If an unscheduled visit occurs, a member of the clinical study team (PI, subinvestigator, nurse coordinator, or clinical nurse) will interview and evaluate the subject to determine the cause of the visit and provide care as needed.

7 Management of Expected Norovirus Illness

7.1 Clinical Evaluation

After NoV challenge, subjects will remain on the ward for at least 5 days. Vital signs (temperature, blood pressure, heart rate, breathing rate) will be measured every 2 hours during waking hours over the first 2 days and at least every 8 ± 1 hour thereafter by staff. Subjects will be interviewed daily by a study Investigator to determine the occurrence of illness signs and symptoms (e.g., diarrhea, vomiting, headache, nausea, fever, abdominal cramps or pain, abdominal gurgling or bloating, myalgia) which occurred during the previous study day; these 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.

7.2 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 stool, as follows:

- Grade 1 – well formed (normal stool, does not take the shape of the container)
- Grade 2 – soft (normal stool, 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 (diarrheal stool) must be weighed, 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.

7.3 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 (BUN, and creatinine).

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

7.4 Indications for Concomitant Medications

Any concomitant medication, prescription or over-the-counter, will be evaluated for continuation during the inpatient setting (e.g., 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 (i.e., ibuprofen, acetaminophen, aspirin, or similar non-steroidal agents) may be prescribed for severe headaches, other pains, or fevers (e.g., sustained temperatures of $\geq 39°C$).
- At the investigator's discretion (e.g., 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 ASSESSMENT OF SAFETY

The assessment of the safety of the study drug and challenge agent will be through the detection and documentation of AEs, both solicited AEs and unsolicited AEs, and/or clinically significant laboratory abnormalities, through 28 days post-challenge. Only the occurrence of SAEs will be reported between Days 28 and 45.

8.1 Definition of Adverse Event

An AE is any untoward medical occurrence in a subject after administration of the investigational drug and that does not necessarily have a causal relationship with the investigational drug. 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 drug, whether related to the investigational drug. 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 AEs that could potentially be in association with NoV infection. Investigators will attempt to assign causality of solicited AEs to NoV infection or an alternate cause. The pre-specified solicited signs or symptoms will be captured in the designated collection tool for solicited AEs up until Study Day 9. After this time point, all AEs (unsolicited) will be captured on the AE log.

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. Investigators will attempt to assign causality of solicited AEs to NoV infection or an alternate cause.

8.1.1 Grading of Severity of an Adverse Event

Severity of AEs: All AEs will be assessed by the clinician using a protocol-defined grading system. For events not included in the protocol-defined grading system, the following guidelines will be used to quantify severity:

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.

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

Life threatening: any adverse drug experience that places the subject, in the view of the investigator, at immediate risk of death from the reaction as it occurred, i.e., it does not include a reaction that had it occurred in a more severe form, might have caused death.

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.

8.1.2 Relationship to Challenge Dose

Relationship (causality or attribution) of all AEs to NoV 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 NoV 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 NoV 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 NoV and there is a reasonable alternate etiology.

8.2 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 Investigator 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 deaths and immediately life-threatening events, 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.

8.3 Study Halting Rules

The study plans to have 2 cohorts of subjects challenged. After the first cohort is challenged there will be a safety assessment to ensure halting criteria are not present. All subsequent study subjects will continue to be actively monitored and managed for their clinical illness (if during the inpatient period) and continued to be followed for safety information (if during the outpatient period).
The halting rules refer to suspected adverse reactions and will be triggered automatically if any of the events described below are met during the conduct of the study:

- One or more subjects experience a 'related' SAE
- Two or more subjects experience a (grade 3) systemic adverse events and/or two or more (grade 3) non-GI adverse events of the same type regardless of causality, within 3 days after challenge. Refer to section 1.4.1.1 of the protocol for known potential risks with the challenge NV Lot 001-09NV.

8.4 Safety Oversight

An Independent Safety Monitor (ISM) will perform the oversight of safety for this study. The ISM is a scientist or clinician that is not involved with the conduct of the study. The primary responsibility of the ISM is to monitor subject safety. The ISM considers study-specific data as well as relevant background information about the disease, test agent, and target population under study. The ISM will review challenge data shortly after completion of the inpatient phase. The ISM is empowered to suspend the study, recommend amendments to the protocol, and/or to request further information.

The ISM will be responsible for reviewing the cumulative safety data, including a review of safety laboratory test results and AE reporting, should there be a halt in the study. If the study is halted, no further challenges will be performed; all enrolled subjects will continue to be followed, the management of diarrhea or other symptoms will continue until resolution.

9 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.

- Monitoring for this study will be managed by the IND Sponsor
- Vaxart will be provided copies of monitoring reports within 30 days of visit.
- 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.

10 STATISTICAL CONSIDERATIONS

10.1 Hypotheses

This is not a hypothesis-driven study and thus no formal hypothesis testing is planned. The purpose of this study is to verify that NV strain Lot 001-09NV is infectious and able to cause illness. A higher and moderate inoculum dose will be studied. The dose that is both safe and able to induce illness in the majority of subjects will be chosen for use in vaccine evaluation studies.

10.2 Sample Size Considerations

The sample size chosen for this study is not based on power calculations, but rather based on appropriateness for a challenge strain that has not been administered to human subjects. The study plans to challenge 8 subjects to a moderate dose of the challenge strain before challenging 8 subjects at a higher dose of the challenge strain, for a total of 16 subjects challenged. **Table 7** illustrates the probability (%) of observing one or more events of any specific type given hypothetical "true" event rates. While these results could be applied to any event rate that follows a binomial distribution, they are included primarily to understand the probability of observing one or more AE or SAE given the number of subjects.

Table 7: Power (%) to Detect an Event

Event Frequency	N=8	N=16
0.01%	0.080	0.160
0.10%	0.797	1.588
1.00%	7.726	14.854
10.00%	56.953	81.470

10.3 Analysis

The primary endpoint is to evaluate the safety of the human challenge model with NV inoculum as measured by the number infected, number that become ill, and the number of SAEs. Because the numbers of subjects are small, descriptive statistics will be used for the following:

- Number (%) infected and ill by challenge dose
- Symptom profile and symptom severity (no./%) by dose
- Number (%) of SAEs and SAEs by dose

Secondary:

- Immune response to NV
  - Serum NV specific IgA and IgG by dose, at Day 0 and Day 28

In the ELISA to determine anti-NV IgG seroconversion will be performed. Each specimen is tested at a single dilution (1:400), and the IgG titer is calculated relative to the standard curve of the positive control. Specimens that do not give a signal in the linear part of the standard curve will be retested at a higher (1:2000) or lower (1:100) dilution as appropriate. Each specimen is tested in duplicate positive (coated with NV VLPs) and negative (coated with buffer) test wells in order to control for the effect of background signal for each individual specimen.

Seroconversion is defined as a 4-fold or greater rise in IgG titer compared to the IgG titer in the prechallenge specimen. A sample of 1µl of sera is sufficient for this assay. A negative control includes a blank well with no antigen but assayed for binding of anti-NV antibodies.

- Determine the length of viral shedding in stool and emesis samples after challenge.
- Quantitate the level of viral shedding by dose

Exploratory:

- Number (percent) of subjects with at least a 4-fold rise in NV-specific antibody titers post challenge.
- Number (percent) of subjects that seroconvert post-challenge, subdivided by illness status.
- Number (percent) of subjects with at least a 4-fold rise in HBGA BT_{50} from baseline compared to Day 28
- ASC IgA and IgG, Day 1 and Day 6 and/or Day 9
- Fecal IgA and saliva IgA, pre-challenge to post-challenge

11 SOURCE DOCUMENTS AND ACCESS TO SOURCE DATA/DOCUMENTS

11.1 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' memory aid or 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 Case Report Form (CRF) will be traceable to these source documents, which are generally maintained in the subject's study file. The source documents will include a copy of the signed Informed Consent/ Health Insurance Portability and Accountability Act (HIPAA) authorization. The source document data collection forms for Screening, Inpatient Pre-Challenge to Day of Challenge, Inpatient Post-Challenge, Flow Sheet of Stool and Emesis Record, Outpatient Visits and AEs will also serve as CRF data collection instruments.

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.

11.2 Data Management

Source data verification of all key safety, laboratory, and clinical data in the database will be conducted by the study monitor after data entry has been completed by the site staff. Coexistent medical conditions, AEs and other medical events will be coded using MedDRA dictionary. Concomitant medications will be coded using WHO-Drug dictionary. 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.

12 GOOD CLINICAL PRACTICE COMPLIANCE

- This study will be conducted in accordance with the protocol and with the following:
  - Consensus ethical principles derived from international guidelines including the Declaration of Helsinki and Council for International Organizations of Medical Sciences (CIOMS) International Ethical Guidelines
  - Applicable ICH Good Clinical Practice (GCP) Guidelines
  - Applicable laws and regulations

13 ETHICAL CONSIDERATIONS (AND INFORMED CONSENT)

13.1 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 Code of Federal Regulations (CFR) Part 46 and/or the International Conference on Harmonisation (ICH) E6; 62 Federal Regulations 25691 (1997).

13.2 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.

Prior to enrollment of subjects into this clinical study, the protocol and the informed consent form(s) 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. Should amendments to the protocol be required, the amendments will be submitted to the IRB; an IRB letter of approval of the amendment must be obtained prior to acting upon the amendment in the protocol.

13.3 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 separate Informed Consent Forms (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. 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.

13.4 Subject Confidentiality

Subject (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 subject.

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 monitor 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 each clinical site. The study database will be user-restricted and password-protected. The study database will identify subjects by a coded study Subject ID number assigned by clinical site personnel, thus subjects will not be identified by name.

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APPENDIX A: PROTOCOL REVISIONS

Version	Date	Significant Revisions	Ethics/Regulatory Approvals
1.1	21 Sept 2018	Removed Section 1.4.1.4, Rectal swab Modified list of systemic solicited signs or symptoms Modified Section 8.3, Study Halting Rules Added Adverse Events following Challenge to Appendix C Modified Appendix D	

APPENDIX B: CHALLENGE VIRUS

Preparing Norovirus Inoculum According to cGMP

The facility that was used to manufacture the inoculum is in the Michael Hooker Research Center at the University of North Carolina in Chapel Hill, NC. 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 (**Table 8**). 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 8: 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 cGMP is maintained through a quality systems approach. This approach involves developing specific quality control programs to address each of the regulatory requirements of Good Manufacturing Practice (GMP). The specific quality control and quality assurance programs that are used to maintain cGMP are listed in **Table 9** 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 9: 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 (WuXi-Apptec) 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 certificate of analysis 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 10** and **Table 11**).

Table 10: 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 Wu-Xi Apptec Protocol #30744,30736, and 30751	Negative	Negative
Mycoplasma	Points to consider for Viral Stocks, GLP Wu-Xi Apptec Protocol # 30200	Negative	Negative
Endotoxin	BET/LAL Gel Clot I/A Assay, GMP Wu-Xi Apptec Protocol # 30739	< 500,000 EU/mL	3640 EU/mL
In Vitro Adventitious Virus	GLP, 3 Cell Lines, 28 days Wu-Xi Apptec Protocol # 30625	Negative	Negative
In Vivo Virus	Adult and Suckling mice, Eggs Wu-Xi Apptec 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 11: 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 Wu-Xi Apptec Protocol #30744, 30751 and 30736	Negative	Negative
Mycoplasma	Points to consider for Viral Stocks, GLP Wu-Xi Apptec Protocol # 30200	Negative	Negative
Endotoxin	BET/LAL Gel Clot I/A Assay, GMP Wu-Xi Apptec Protocol # 30743	< 5000 EU/mL	235 EU/mL

APPENDIX C: SCHEDULE OF STUDY VISITS AND EVALUATIONS

	Pre-Screening visit	Screening visit	Challenge phase (Inpatient)						Follow up Visits			
Study Day	-60 to -Screening	-30 to -3	1 Pre	2 Chal	3	4	5	6 /Disc	9	14	28	45
Compliance Range									± 1 d	± 3 d	± 3 d	± 3 d
Visit	00A	00B	00C	1	2	3	4	5	6	7	8	9
Outpatient	X	X							X	X	X	X
Inpatient stay			X	X	X	X	X	X				
Informed Consent	X	X										
ABO blood typing	X											
Saliva test for secretor status	X											
Demographics	X	X										
Weight, Height, BMI	X	X	X	X	X	X	X	X				
Eligibility criteria	X	X										
Medical interview		X	X	X	X	X	X	X	X	X	X	X
Physical Exam, (X) = targeted		X	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	
Vital signs		X	X	X	X	X	X	X	X	X	X	
Concomitant medications		X										
ECG		X										
Screen for HIV, HBsAg and HCV		X										
Safety laboratory evaluation		X	X					X				
Drug screen (urine, alcohol breath)		X	X									
Stool sample for enteric pathogens and occult blood		X										
Pregnancy test (females)		X	X								X	
Challenge				X								
Symptom grading				X	X	X	X	X				
Adverse events				X	X	X	X	X	X	X	X	X
Stool weighing/grading				X	X	X	X	X	X	X	X	
Emesis weighing				X	X	X	X	X				
Norovirus shedding (fecal/emesis)				X	X	X	X	X	X	X	X	
Discharge from inpatient								X				
Diary card (issue and/or return)								X	X			
Phone interview												X
Immunology												
Serum sample (antibody)			20 ml								20 ml	
Whole blood sample (PBMCs)			60 ml					60 ml	60 ml			
Fecal sample (IgA)			X								X	
Saliva sample (IgA)			X								X	
Assay Description												
qRT-PCR, Fecal	-	-	X	X	X	X	X	X	X	X	X	
qRT-PCR, Emesis	-	-		X	X	X	X	X				
VP1 IgG/IgA ELISA, Serum	-	-	X								X	
Assay Description (Exploratory)												
BT50, Serum	-	-	X								X	
ASC (IgG+IgA), PBMC	-	-	X					X	X			
VP1 IgA ELISA, Fecal	-	-	X								X	
VP1 IgA ELISA, Saliva	-	-	X								X	

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

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

APPENDIX D: ADVERSE EVENT GRADING SCALES

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

Systemic 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)	
Solicited symptoms include: headache, nausea, abdominal cramps or pain, abdominal gurgling or bloating, myalgia	No interference with activity	Some interference with activity	Prevents daily activity	Requires hospitalization

APPENDIX E: 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