Phase I Study to Determine the Optimal Human Challenge Dose for Norovirus GII.4 CIN-3 Batch No.: 01-16C3

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STATEMENT OF ASSURANCE

Each Institution will hold a current Federal Wide Assurance (FWA) issued by the Office of Human Research Protections (OHRP) for federally-funded human subjects research. Each FWA will designate at least one Institutional Review Board (IRB)/Independent Ethics Committee (IEC) registered with OHRP, for which the research will be reviewed and approved by the IRB/IEC and will be subject to continuing review [45 CFR 46.103(b)]. The IRB/IEC designated under an FWA may include an institution's IRB/IEC, an independent IRB/IEC, or an IRB/IEC of another institution after establishing a written agreement with that other institution.

STATEMENT OF COMPLIANCE

The study trial will be carried out in accordance with Good Clinical Practice (GCP) and as required by the following:

- United States Code of Federal Regulations (CFR) 45 CFR Part 46: Protection of Human Subjects
- Food and Drug Administration (FDA) Regulations, as applicable: 21 CFR Part 50 (Protection of Human Subjects), 21 CFR Part 54 (Financial Disclosure by Clinical Investigators), 21 CFR Part 56 (Institutional Review Boards), 21 CFR Part 11, and 21 CFR Part 312 (Investigational New Drug Application), 21 CFR 812 (Investigational Device Exemptions)
- International Conference on Harmonisation: Good Clinical Practice (ICH E6); 62 Federal Register 25691 (1997); and future revisions
- Belmont Report: Ethical Principles and Guidelines for the Protection of Human Subjects of Research, Report of the National Commission for the Protection of Human Subjects of Biomedical and Behavioral Research
- National Institutes of Health (NIH) Office of Extramural Research, Research Involving Human Subjects, as applicable
- National Institute of Allergy and Infectious Diseases (NIAID) Clinical Terms of Award, as applicable
- Applicable Federal, State, and Local Regulations and Guidance

SIGNATURE PAGE

The signature below provides the necessary assurance that this trial will be conducted according to all stipulations of the protocol, including all statements regarding confidentiality, and according to local legal and regulatory requirements and applicable US federal regulations and ICH E6 Good Clinical Practice (GCP) guidelines.

I agree to conduct the study in compliance with GCP and applicable regulatory requirements.

I agree to conduct the study in accordance with the current protocol and will not make changes to the protocol without obtaining the sponsor's approval and IRB/IEC approval, except when necessary to protect the safety, rights, or welfare of subjects.

Site Investigator Signature:

Signed: _____ Date: _____

Name

Title

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

AE	Adverse Event
AGE	Acute Gastroenteritis
ALS	Antibody Lymphocyte Supernatant
ALT	Alanine Aminotransferase
ANC	Absolute Neutrophil Count
APC	Antigen Presenting Cell
ASC	Antibody-Secreting Cell
BPM	Beats Per Minute
BUN	Blood Urea Nitrogen
°C	Degrees Celsius
CCHMC	Cincinnati Children's Hospital Medical Center
CDC	Centers for Disease Control
CMS	Clinical Material Services
CFR	Code of Federal Regulations
CONSORT	Consolidated Standards of Reporting Trials
CRF	Case Report Form
DC	Dendritic Cell
DCC	Data Coordinating Center
DNA	Deoxyribonucleic Acid
DSMB	Data and Safety Monitoring Board
EBV	Epstein Barr Virus
eCRF	Electronic Case Report Form

EIA	Enzyme Immunosorbent Assay
ELISA	Enzyme-Linked Immunosorbent Assay
°F	Degrees Fahrenheit
FDA	Food and Drug Administration
FWA	Federal wide Assurance
GII.4	Norovirus genogroup II, genotype 4
GCP	Good Clinical Practice
GM-CSF	Granulocyte-Macrophage Colony Stimulating Factor
HBGAs	Histo-blood Group Antigens
HEENT	Head, Eyes, Ears, Nose, Throat
Hgb	Hemoglobin
HID50	Human Infectious Dose Causing 50% Infection
HIV	Human Immunodeficiency Virus
HRP	Horseradish Peroxidase
ICF	Informed Consent Form
ICH	International Conference on Harmonisation
ICS	Intracellular Cytokine Staining
IDES	Internet Data Entry System
IEC	Independent or Institutional Ethics Committee
IgA	Immunoglobulin A
IgG	Immunoglobulin G
IL	Interleukin
IND	Investigational New Drug Application

IRB	Institutional Review Board
ISM	Independent Safety Monitor
IUD	Intrauterine Device
MedDRA ®	Medical Dictionary for Regulatory Activities
MOP	Manual of Procedures
N	Number (typically refers to subjects)
NK	Natural Killer Cell
NOCMCs	New-Onset Chronic Medical Conditions
NoV	Norovirus
NPO	Nothing by mouth
NV	Norwalk Virus
OHRP	Office for Human Research Protections
ORS	Oral Rehydration Solution
OTC	Over the Counter
PBMC	Peripheral Blood Mononuclear Cell
PEG	Polyethylene Glycol
PP	P-Particle
QA	Quality Assurance
QC	Quality Control
qRT-PCR	Quantitative Reverse Transcriptase Polymerase Chain Reaction (also known as Real-time RT-PCR)
RNA	Ribonucleic Acid
RT-PCR	Reverse Transcriptase Polymerase Chain Reaction

SAE	Serious Adverse Event
SMC	Safety Monitoring Committee
SOP	Standard Operating Procedure
ULN	Upper Limit of Normal
US	United States
USP	United States Pharmacopeia
VLP	Virus-Like Particle
WBC	White Blood Cell

PROTOCOL SUMMARY

Title:

Phase I Study to Determine the Optimal Human Challenge Dose for Norovirus GII.4 CIN-3 Batch No.: 01-16C3

Design of the Study:

Subjects who meet eligibility during the screening period (Day -60 to -2) will be admitted to an inpatient unit (the Vaccine Research Center inpatient facility) and have a COVID-19 antigen test performed. Participantswill be admitted the day prior to challenge. Participants will be kept in their room until the test result is available. Participants with a negative result will be eligible for study continuation while anyone with a positive COVID-19 test will be immediately escorted out of the unit. The day after admission to the inpatient unit, eligible participants will be challenged with a dose of human Norovirus GII.4 CIN-3 Batch No.: 01-16C3.
The challenge study will be conducted in 3 separate cohorts of approximately 16 subjects each, with the initial cohort receiving $3.5x10^3$ genome copies of norovirus. As this is an established challenge model using another batch of the virus used previously, all subjects in a cohort will be given the challenge dose at the same time. For every 16 subjects, 15 will be chosen because they have a functional fucosyl transferase-2 (FUT-2) gene (secretor positive) and 1 will lack a functional FUT-2 gene (secretor negative). Based on the illness rate of acute gastroenteritis (AGE) in the first cohort, the second cohort, and subsequent cohorts, will receive higher or lower doses based on a pre-specified decision tree (as detailed in Figure 1).

At the time of the cohort admission, 2-3 additional subjects will be admitted and serve as alternates. The alternates would become primary study subjects if a subject in the primary group is unavailable or becomes ineligible at the time of the inpatient study. If alternates are not needed, they will be sent home before challenge and offered to be a primary subject in the next cohort. Alternate subjects will be re-screened if their screening information falls outside the protocol designated window for a subsequent cohort.

Subjects will remain in the inpatient facility for at least four days following challenge and discharged only upon meeting discharge criteria in Section 6.1.3. Subjects will be assessed daily for clinical

	and virologic evidence of norovirus infection. Subjects will return to the investigational site for evaluation on Day 6 (6-8 days), Day 15 (14-16 days), Day 30 (28-35 days), Day 45 (40-50 days), and Day 60 (55-65) post challenge for evaluation of safety, virus shedding and clinical evaluations as detailed in Section 7, Table 1 and Appendix A, schedule of events. A final study contact will be performed on Day 180 (Day 166-194) to obtain an interim medical history and capture of SAEs that have occurred since the last visit will be solicited.
Study Phase:	Phase I
Study Population:	48 generally healthy adults, 18-49 years inclusive
Number of Sites:	1
Description of Study Product or Intervention:	GII.4 norovirus oral challenge
Study Objectives:	**Primary** Determine the optimal challenge dose of Norovirus GII.4 CIN-3 Batch No.: 01-16C3 norovirus to achieve illness in ≥50% of subjects (illness is defined as norovirus infection determined by positive PCR and either a) ≥3 loose or liquid stools in a 24-hour period, b) ≥300 gm of loose or liquid stool in a 24-hour period c) and/or any episode of vomiting), during the inpatient period. **Secondary** • Evaluate the safety of the Norovirus GII.4 CIN-3 Batch No.: 01-16C3 challenge strain • Determine the rate of infection at different challenge doses by:

- Detection of norovirus GII.4 in the stool using specific qRT-PCR
- Anti- norovirus GII.4 serum IgG by ELISA (≥4-fold rise from baseline to Day 30)

- Measure the severity of acute gastroenteritis
- Determine the quantity and duration of virus shedding in stool by qRT-PCR

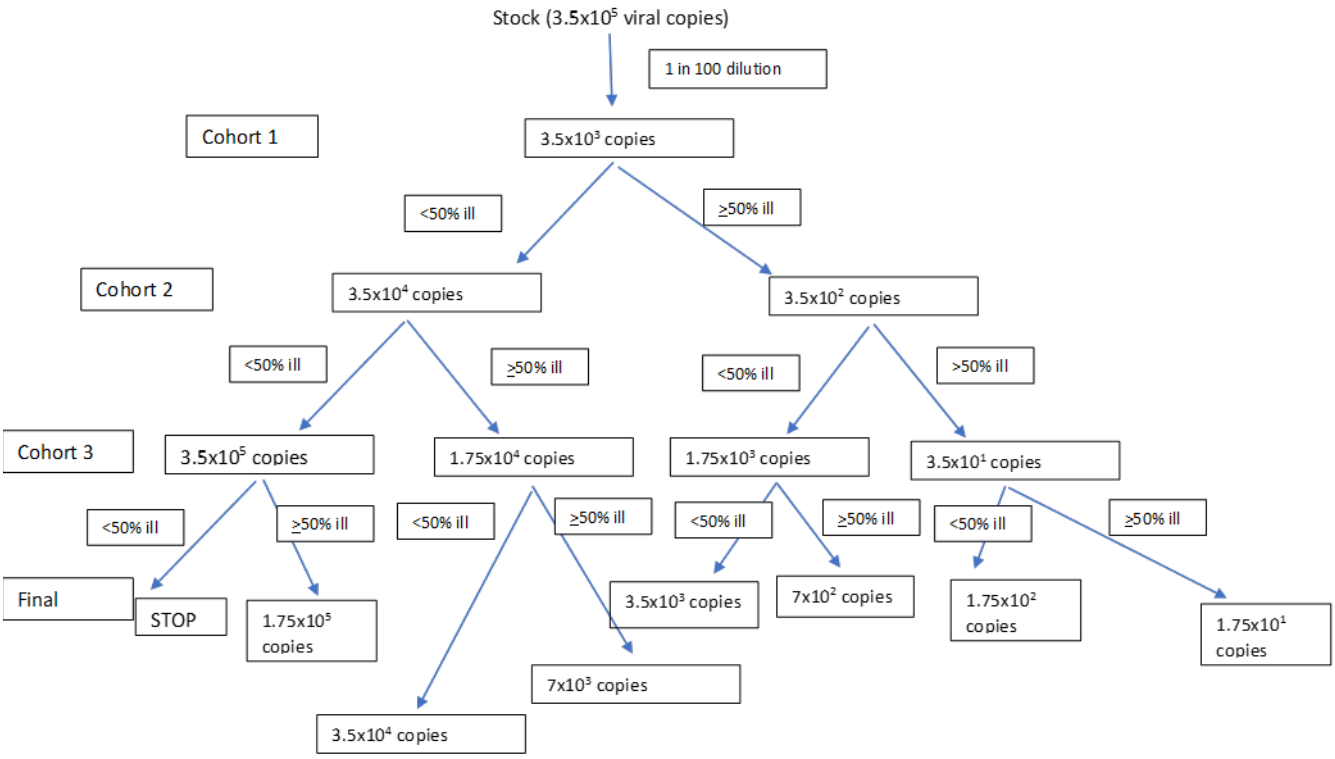
Exploratory

- Determine norovirus GII.4 -specific antibody responses at baseline, Day 15 and Day 30
  - Serum IgA and IgG by ELISA
  - Serum Blocking Antibody by ELISA
- Determine total and norovirus GII.4 -specific Memory B cell response by ELISpot assay
- Determine the effect of baseline norovirus antibody levels (serum IgG, IgA, and blocking antibody) on becoming infected with norovirus
- Determine total and norovirus GII.4 -specific IgA- and IgG-Antibody Secreting Cells by ELISpot assay and Antibody Lymphocyte Supernatant by ELISA

Future Use: Samples of blood (serum and PBMCs) and stool will be collected at various time points as listed on the schedule of events. The samples will be processed and archived for future testing.

Duration of Individual Subject Participation:	6-8 months (screen, inpatient challenge and outpatient follow-up)
Estimated Time to Last Subject/Last Study Day:	Approximately 12-18 months

Figure 1: Schematic of Study Design



"Final" is the dose to be used in the future and not within this protocol.

1 KEY ROLES

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2 BACKGROUND AND SCIENTIFIC RATIONALE

2.1 Background

Norovirus (NoV) is a family of single-stranded, positive-sense RNA (Ribonucleic Acid) viruses that are a leading cause of gastroenteritis worldwide as well as the leading cause of foodborne-associated diarrheal outbreaks in the United States. The Centers for Disease Control (CDC) estimates that on an annual basis NoV is associated with 267M cases globally and 21M cases in the United States with 71,000 U.S. hospitalizations. NoV is particularly problematic in the extremes of age. On a yearly basis, throughout the world, NoV causes over 200K deaths/year, especially in children less than 5 years of age and is a common cause of ER visits and hospitalization among children in the United States[1]. In the US, NoV is estimated to be the cause of death of approximately 800 people over 65 years of age per year. All age groups are susceptible to NoV-related disease. Part of the reason is the diversity of NoV. Three different genogroups (GI, GII, and GIV) are capable of infecting humans, with ≥25 genotypes, and many subgroups[2]. Recombination of related viruses and error-prone RNA replication have contributed to the wide diversity of NoV[2]. Outbreaks usually have high attack rates and have occurred in childcare centers, schools, restaurants, summer camps, hospitals, nursing homes, ships (both civilian and military) as well as deployed military troops. The minimal infectious dose may be as low as 100 virions, which may be passed in foods, beverages, by person-to-person contact, via aerosols from vomit, and in contaminated ground waters[3]. NoV disease occurs throughout the year, though major outbreaks tend to occur during winter months.

NoV are difficult to study because: 1) they are genetically, and antigenically highly diverse and multiple strains co-circulate in the same communities, making diagnosis and disease control extremely difficult; 2) the virus cannot be cultivated long term in cell culture; and 3) no small animal model exists for studying human NoV. Several animals have been tested as potential models, including macaques, chimpanzees, and gnotobiotic pigs. Although chimpanzees can be infected with human NoV, they do not become ill[4]. Therefore, humans represent the only complete disease model for studying human NoV.

As not everyone gets infected or ill when exposed, it has been determined that susceptibility to NoV is modulated in part by specific, genetically determined carbohydrates in the gut known as the Histo-blood Group Antigens (HBGAs)[5]. These carbohydrates are found on the surfaces of red blood cells and mucosal epithelia. These antigens are also found circulating as free oligosaccharides in milk, saliva, and blood. Approximately 80% of humans (termed secretors)

have a functional fucosyl transferase-2 (Fut-2) gene that leads to expression of HBGAs to which many NoVs can adhere. The remainder of the population lack a functional Fut-2 gene and are referred to as "non-secretors". Only secretor-positive individuals can be infected with Norwalk Virus (NV), a genogroup 1 virus, supporting the theory that the H type 1 antigens are the cellular receptors for NV. Other HBGAs can also function as receptors, and different norovirus genogroups exhibit unique patterns of HBGA binding[6]. Additionally, challenge studies with Snow Mountain virus (GII.2) and outbreak studies with other GII viruses have reported infections in secretor negative individuals, suggesting the use of receptors other than the secretor-associated HBGAs[7,8].

A GII.4 human norovirus called CIN-1 has been used previously for challenge studies. We have demonstrated that CIN-1 is infectious and induces a transient clinical illness of mild to moderate severity[9]. Forty healthy adults (23 secretors (predicted high susceptibility to infection) and 17 non-secretors (predicted low susceptibility to infection) with low serum antibody levels to GII.4 (ELISA IgG ≤1:1600) were administered an oral dose of $4.4x10^4$ RT-PCR units (equivalent to $3.5x10^5$ genome copies/mL) of CIN-1. Of the 23 secretors, 16 (70%) were infected with norovirus, 13 (57%) became ill (characterized by vomiting and/or diarrhea), and 12 (52%) developed norovirus-associated illness. In contrast, only 1 non-secretor (5.9%) became ill but did not have norovirus identified from the stool, and another non-secretor shed virus for a single day but was asymptomatic (P <.001 for each variable, compared with secretors). Infection occurred in secretors regardless of ABO blood group[9].

A second protocol was completed recently at Cincinnati Children's Hospital Medical Center (CCHMC) to determine whether a lower dose of CIN-1 would induce infection (unpublished). A total of 38 healthy, adult subjects (34 secretors and 4 non-secretors) with low serum antibody levels to GII.4 (ELISA IgG ≤1:1600) were administered $4.4 x10^3$ RT-PCR units of CIN-1 (unpublished data). Of the 34 secretors, 26/34 (76%) had norovirus detected in the stool by RT-PCR testing and/or a fold-fold rise in antibodies against GII.4 norovirus (unpublished data). In contrast, none of the 4 non-secretors had evidence of infection. The percentage of subjects who were secretors and developed infection after administration of CIN-1 at $4.4 x10^3$ RT-PCR units was equivalent to the rate of infection among subjects who received $4.4 x10^4$ RT-PCR units in the first study. However, neither study explored whether the further lowering of the dose is possible without decreasing the rate of infection[10,11].

2.2 Scientific Rationale

The initial outbreak of norovirus in Norwalk, Ohio was a GI.1[12]. This isolate has been stored at the NIH as well as Baylor College of Medicine (Houston, Texas). The isolate has been used in clinical trials and the dose needed for the human infectious dose 50% (HID_{50}) has been determined[13]. This isolate also has been used in a challenge model to evaluate a genogroup I virus-like particle (VLP) vaccine[14]. However, currently, most NoV infections are due to genogroup II viruses and there is thought to be little to no cross-protection between genogroups.[2,15]. Therefore, the GI.1 challenge data cannot be used for genogroup II vaccine development. As described above, GII.4 NoV have been used in challenge models but the lowest dose capable of inducing infection in 50% of the susceptible subjects has not been determined. This information is critical to the testing of agents designed to prevent or treat NoV. Concerns are that using a dose of NoV in excess of what a subject typically would encounter in nature could result in inappropriate rejection of a promising vaccine or therapeutic. To facilitate these studies, dose range studies for both infection and illness for new GII inoculums must be completed. Once the shape of the dose-response relationship has been characterized, the optimal sample size and most efficient challenge doses needed for future trials can be accurately determined, limiting the expense, doses utilized, and health risk of these trials. By determining the dose-response relationship, future vaccine and therapeutic studies can make most efficient use of valuable challenge strains (i.e., a critical dose-sparing efficiency). Furthermore, with well-characterized challenge strains, we can more accurately estimate the efficacy of the vaccine or therapeutic treatment with minimal error in the estimate of the predicted infection rate without the vaccine.

As discussed above, for CIN-1 (GII.4) both a $4.4x10^4$ RT-PCR unit and $4.4x10^3$ RT-PCR unit dose resulted in approximately 70% of susceptible subjects being infected and nearly 60% becoming ill following challenge[9] (and unpublished). However, these doses still exceed that used in other challenge studies and may exceed the amount that occurs with natural exposure. Therefore, this higher titer may not be appropriate for vaccine evaluations. Thus, further study to evaluate decreases in dose levels is needed. Quantification of the prior CIN-1 challenge strain with a titer of $4.4x10^4$ RT-PCR units/mL resulted in an updated titer of $3.5x10^5$ copies/mL. Based on the rate of infection and disease (infections plus symptoms including vomiting and/or diarrhea) in the initial cohort; the dose for the second cohort will be selected. Similarly, based on the response in the second cohort, the dosing for the 3rd cohort will be determined.

2.2.1 Purpose of Study

Determine the minimum dose of Norovirus GII.4 CIN-3 Batch No.: 01-16C3 required to produce virus-associated illness in ≥50% subjects.

2.2.2 Study Population

Healthy, non-pregnant adults, 18-49 years inclusive, negative for COVID-19 by antigen testing at the time of norovirus challenge, will be enrolled into this study.

Pregnant women will be excluded for safety purposes.

2.3 Potential Risks and Benefits

2.3.1 Potential Risks

Blood Collection/Intravenous Catheter Placement

Drawing blood may cause transient discomfort and may cause people to feel faint. Bruising at the blood draw site may occur but can be prevented or lessened by applying pressure for several minutes. Infection at the site of blood collection is a rare but possible event. If required for intravenous fluid hydration, placement of an intravenous catheter may be associated with mild pain, redness and bruising which may take up to 10-14 days to resolve. Rarely, infection or a blood clot may develop. Risks of blood collection and intravenous catheter placement will be minimized by using sterile technique and having experienced personnel perform the blood collection.

Receipt of 2% sodium bicarbonate solutions

Based on previous experience with NoV challenges, sodium bicarbonate solution used to buffer the stomach before and after administration of the challenge strain will taste salty.

Receipt of Study Agent (NoV GII.4 Challenge Strain)

Based on prior GII.4 studies, after receipt of a dose of NoV, it is expected that up to 70% of susceptible subjects (secretors) will become infected (i.e. shed NoV in their stool) and 50-60% of secretors will develop disease (vomiting, diarrhea). In our experience of over 100 people who have been administered GII.4 CIN-1; symptoms typically begin after a 12-48 hour incubation period after ingestion of the NoV challenge. Common symptoms have been nausea, abdominal cramps, vomiting and diarrhea. The frequency of vomiting and diarrheal episodes experienced by

subjects is variable. However, most have had mild to moderate symptoms and resolve within 1-2 days.

While recurrent or prolonged vomiting and diarrhea may lead to dehydration, every subject in our previous studies has been managed successfully with oral rehydration solution (ORS) without need of intravenous fluids.

Although not known to occur, it is possible that subjects could have a hypersensitivity reaction to the challenge strain material. Since NoV challenge virus strains cannot be produced in a laboratory by viral culture, the challenge NoV strains are made from human stool filtrates with a known viral concentration. The GII.4 challenge to be used in this study was isolated in 2011 from a stool sample collected as part of our inpatient challenge model of norovirus (Subject 02CCI 0249). The donor was tested and negative for anti- HAV IgM, HBsAg, anti-HCV as well as HIV (Human Immunodeficiency Virus). He also had stool culture for routine enteric pathogens and stool ova and parasite examination, again, all of which were negative. However, despite the careful testing of the product, there is a slight risk of exposure to other infectious agents when subjects are administered the challenge product.

Peri-anal Dermatitis

Another possible risk following challenge is peri-anal dermatitis, as a result of diarrhea.

Spread of the NoV Challenge Strain

To minimize the likelihood of transmission of virus to study staff from a subject, study staff will use good hand hygiene procedures while in the inpatient facility and wear gowns and gloves for activities involving contact with subjects. Face masks will be worn if the subject is actively vomiting.

Subjects will be advised to wash their hands frequently, especially after toilet use and before meals. Subjects will receive counseling and education about the acute gastroenteritis associated with NoV and the necessary precautions to minimize the potential for spread of disease to others prior to discharge. At the time of discharge from the inpatient facility, subjects may still be shedding NoV in their stools. The exclusion criteria for subjects for this research study have been chosen to minimize the potential for a subject to transmit NoV to those at highest risk of severe disease (e.g., the elderly, young infants, and immunocompromised individuals)[9,13].

Acquisition/Spread of COVID-19

The pandemic of COVID-19, which began in Wuhan, China in December 2019, continues as of the time of the amended protocol (October 2021). To minimize the risk of participants acquiring and/or spreading COVID-19, all participants will have a negative test for COVID-19 at the time of admission to the inpatient unit. Upon arrival at the inpatient unit, participants immediately will be escorted to their room. Participants will be instructed to remain in their room until the result of the COVID-19 test is available. Any participant found to have a positive COVID-19 test will be escorted out of the inpatient unit and their participation in that cohort will be completed. However, if interested, such participants still could enroll in a subsequent cohort.

2.3.2 Potential Benefits

There is no known benefit to the subject. However, the results of the study could benefit society by better understanding NoV infection and characterizing a dose of NoV to be used in future trials testing candidate vaccines and/or therapeutics.

3 STUDY DESIGN, OBJECTIVES AND ENDPOINTS OR OUTCOME MEASURES

3.1 Study Design Description

This is a double-blind, safety and infectivity study of experimental human Norovirus genogroup GII.4 administered to healthy non-pregnant adults, 18-49 years of age, inclusive.

Subjects will be admitted to an inpatient unit and then challenged with a dose of human norovirus GII.4 challenge strain. Subjects will be housed in the Vaccine Research Center inpatient facility and administered the dose according to cohort. The challenge study will be conducted in 3 cohorts of approximately 16 subjects each, with the initial cohort receiving $3.5x10^3$ copies of norovirus. For every 16 subjects, 15 will be chosen because they have a functional FUT-2 gene (secretor positive) and 1 will be chosen because they lack a non-functional FUT-2 gene (non-secretor). Every subject in a cohort will receive the same dose of norovirus. From previous studies we have conducted with this GII.4 norovirus, only 1 "non-secretor" became infected and the symptoms experienced were very mild. Thus, administration of a dose of a GII.4 norovirus to a non-secretor is in effect administering a placebo.

Based on the illness rate of subjects meeting the primary outcome measure in secretor positive subjects of the initial cohort, a series of decision rules will be implemented (see Section 10.2) with regards to dosing of the second cohort and third cohort.

Subjects will remain in the inpatient facility for at least four days following challenge and assessed daily for clinical and virologic evidence of norovirus infection. Discharge criteria are listed in Section 6.1.3. Subjects will return to the investigational site for evaluation on Day 6 (6-8 days), 15 (14-16 days,), 30 days (28-35 days), 45 (40-45 days), and 60 (55-65 days) post challenge.

Approximately 180 days after receiving virus challenge, a final study contact will be performed to obtain an interim medical history. Adverse events limited to new-onset chronic medical conditions and SAEs that have occurred since the last visit will be solicited.

3.2 Study Objectives

3.2.1 Primary

Determine the optimal challenge dose of Norovirus GII.4 CIN-3 Batch No.: 01-16C3 norovirus to achieve illness in ≥50% of subjects (illness is defined as norovirus infection determined by positive PCR and either a) ≥3 loose or liquid stools, in a 24-hour period, b) ≥300 gm of loose or liquid stool in a 24-hour period c) and/or any episode of vomiting), during the inpatient period.

3.2.2 Secondary

Evaluate the safety of the Norovirus GII.4 CIN-3 Batch No.: 01-16C3 challenge strain

Determine the rate of infection at different challenge doses by:

- Detection of norovirus GII.4 in the stool using specific qRT-PCR
- Anti- norovirus GII.4 serum IgG by ELISA (≥4-fold rise from baseline through Day 30)

Measure the severity of acute gastroenteritis

Determine the quantity and duration of virus shedding in stool by qRT-PCR

3.2.3 Exploratory

Determine norovirus GII.4 -specific antibody responses at baseline, Day 15 and Day 30

- Serum IgA and IgG by ELISA
- Serum Blocking Antibody by ELISA

Determine total and norovirus GII.4 -specific Memory B cell response by ELISpot assay

Determine the effect of baseline norovirus antibody levels (serum IgG, IgA, and blocking antibody) on becoming infected with norovirus.

Determine total and norovirus GII.4 -specific IgA- and IgG-Antibody Secreting Cells by ELISpot assay and Antibody Lymphocyte Supernatant by ELISA

3.2.4 Future Use Samples/Assays

Samples of blood (serum and PBMCs) and stool will be collected at various time points as listed on the schedule of events. The samples will be processed and archived for future testing.

3.3 Study Endpoints or Outcomes Measures

3.3.1 Primary

The occurrence of NoV-associated illness (vomiting, diarrhea, positive PCR) in secretor positive subjects through Day 4 after challenge.

3.3.2 Secondary

The number of subjects with **solicited** adverse events through Day 10.

The number of **unsolicited** serious adverse events reported through Day 180.

The number of **unsolicited** Grade 3 adverse events from challenge to Day 30.

The number of subjects with infection through Day 30 as determined by:

- Detection of norovirus GII.4 in stool by qRT-PCR at Days 2, 3, 4, 5, 6, 15, 30
- ≥4-fold rise from baseline in GII.4-specific antibody titers in serum IgG by ELISA through Day 30

Peak genome equivalent copies/mL of virus in stool as measured by qRT-PCR after challenge through Day 60.

Duration (number of days) of viral secretion as measured by qRT-PCR after challenge through Day 60.

Modified Vesikari score through Day 4.

Duration (hours) of vomiting and/or diarrhea through Day 5.

3.3.3 Exploratory

Number of subjects with ≥4-fold rise from baseline in GII.4-specific antibody titers in serum (IgA, IgG, Blocking Antibody) by ELISA at Day 15 and Day 30.

Baseline NoV GII.4 serum IgG, IgA, and blocking antibody titers in subjects with and without infection.

Norovirus GII.4-specific Memory B cells determined by ELISpot using cryopreserved PBMCs (Ratio of antigen specific spot forming cells / 10^6 PBMC) baseline, Days 30 and 60.

Norovirus GII.4-specific IgA and IgG ASC per total IgA- or IgG-secreting ASCs baseline and at Days 6 and 15.

Norovirus GII.4-specific IgA and IgG ALS by ELISA baseline and at Days 6 and 15.

4 STUDY INTERVENTION/INVESTIGATIONAL PRODUCT

4.1 Study Product Description

As part of the studies where our GII.4 strain was used in human challenges; samples of diarrhea were saved from all subjects who developed diarrhea after receiving the challenge strain. These stool samples were aseptically transferred to storage containers and have been maintained at $\leq$-60^{0}C in a temperature monitored freezer for subsequent use. Due to exhausting the supply of our original GII.4 challenge pool; samples collected from a subject in our challenge studies has been used to prepare a new challenge pool.

The GII.4 challenge to be used in this study was isolated in 2011 from a stool sample collected as part of our inpatient challenge model of norovirus (Subject 02CCI0249). The donor is a male who was 31 years old at the time of sample donation. He had no prior history of hospitalization or serious illness. To determine eligibility for enrollment into the challenge study, the sample donor was tested for anti- HAV IgM, HBsAg, anti-HCV as well as HIV, all of which were negative. He also had stool culture for routine enteric pathogens and stool ova and parasite examination, again, all of which was negative. Testing of his blood pre-challenge demonstrated a normal CBC and AST of 55 international units (IU) (normal range 30-65 IU). The stool donor received a dose of the previous challenge strain (CIN-1) on October 24, 2011. The following day, the donor began to develop diarrhea which lasted 2 days and then resolved spontaneously. During the 2-day illness, the subject had 10 diarrheal stools.

To make the challenge product to be used in the current study; 2 diarrheal stool samples were combined and the final product (Norovirus GII.4 CIN-3 Batch No.: 01-16C3) was prepared by executing a series of processing steps, including dilution of the original donor specimen, centrifugation, filtration, and filling. Aliquots of the unfiltered stool sample (10% dilution of the 2 pooled diarrheal stool samples) was tested for rotavirus, enteric adenovirus, astrovirus, hepatitis A, HIV-1, HIV-2, cytomegalovirus, hepatitis B virus, and hepatitis C virus, as well as *in vitro* and *in vivo* adventitious viral contamination (*in vitro* AA (amino acid) and *in vivo* AA). Samples of the final product, Norovirus GII.4 Norovirus GII.4 CIN-3 Batch No.: 01-16C3, were tested for sterility, identity and potency by quantitative RT-PCR, direct and indirect methods for the detection of mycoplasma, as well as bacterial endotoxin.

The final challenge product Norovirus GII.4 CIN-3 Batch No.: 01-16C3 was aliquoted into sterile 2mL centrifuge tubes with fill volumes of 1-1.5mL at a concentration of approximately $3.5x10^5$ copies/mL. The challenge material is clear to amber in color and is being stored at $\leq$-60^0C in a temperature monitored freezer.

This study product is a live, infectious agent capable of causing symptoms of fever, headache, nausea, abdominal cramps/discomfort/pain, abdominal gurgling, abdominal bloating, myalgia, malaise/fatigue, anorexia/loss of appetite, and chills.

4.1.1 Formulation, Packaging, and Labeling

Norovirus GII.4 CIN-3 Batch No.: 01-16C3

The filled, purified study product is formulated in sterile water for injection, USP in 2mL sterile cryovials, in volumes of 1-1.5mL at a concentration of $3.5x10^5$copies/mL.

The study product will be labeled according to manufacturer or regulatory specifications and include the statement "Caution: New Drug – Limited by Federal Law to Investigational Use"

Diluent - Sterile Water for Injection, USP

The sterile water for injection, USP is non-pyrogenic and contains no bacteriostatic, antimicrobial agent, or added buffer. This product will be used to dilute the Norovirus GII.4 CIN-3 Batch No.: 01-16C3 and will be supplied as a 2 mL single-dose cryovial.

A label with the statement "Caution: New drug -Limited by Federal Law to Investigational Use" will be placed on the immediate package.

Buffer - Sodium Bicarbonate, USP

The Sodium Bicarbonate, USP is a white, odorless, crystalline powder. This product will be used to prepare the buffer.

A label with the statement "Caution: New drug -Limited by Federal Law to Investigational Use" will be placed on the immediate package.

4.1.2 Product Storage and Stability

The temperature of the storage unit must be manually recorded daily (excluding non-business days and holidays, as applicable) and continuously monitored and recorded during the course of this trial per the participating VTEU site standard operating procedures (SOPs), and documentation will be maintained. If the temperature fluctuates outside of the required range, the affected study product(s) must be quarantined at the correct storage temperature and labeled as 'Do Not Use' (until further notice). The participating VTEU site's research pharmacist must alert the site principal investigator and study coordinator, if the temperature fluctuates outside of the required range. In the event the temperature fluctuates outside of the required range, including accidental deep-freezing or disruption of the cold chain, the affected study product(s) must not be administered. The site principal investigator or responsible person should immediately contact the DMID Product Support Team at DMIDProductSupportTeam@niaid.nih.gov for further instructions before any additional study product(s) are administered. Based on the information collected, DMID and/or the manufacturer will determine whether the affected study product(s) can be used. If it cannot be used, the site will receive specific instructions on how to return the affected study product(s) to the DMID CMS or destroy it on site. Additional instructions for quarantine and DMID contact information are provided in the protocol-specific MOP.

Norovirus GII.4 CIN-3 Batch No.: 01-16C3

The filled, purified challenge material, Norovirus GII.4 CIN-3 Batch No.: 01-16C3, is stored at ≤-60°C in a temperature controlled and continuously temperature monitored freezer.

Stability: As norovirus is not cultivatable, nor able to infect other than in a human challenge model; it is not possible to perform typical stability testing. However, the product will be tested for copy units by real-time PCR prior to starting the trial and each year while this protocol is active. While a consistent real-time PCR titer dose not demonstrate the virus is still alive and infectious; based on previous experience of a GI.1 virus being infectious after 30 years of storage; we will assume that the Norovirus GII.4 CIN-3 Batch No.: 01-16C3 is viable and infectious if the viral copy number is within 20% of the copy number from the previous year.

Diluent - Sterile Water for Injection, USP

- The sterile water for injection, USP vials will be stored at 20°C to 25°C (68°F to 77°F). For excursions between 15-30°C (59°F to 86°F), the product can continue to be used (no quarantine) but the site needs to complete the DMID Study Product Support Team Temperature

Excursion Reporting Form and submit to PST. Based on USP guidelines, excursions between 15°C and 30°C are allowed and will not be considered deviations.

Buffer - Sodium Bicarbonate, USP

- The sodium bicarbonate used to prepare buffer, USP container will be stored at 20°C to 25°C (68°F to 77°F). For excursions between 15-30°C (59°F to 86°F), the product can continue to be used (no quarantine) but the site needs to complete the DMID Study Product Support Team Temperature Excursion Reporting Form and submit to PST. Based on USP guidelines, excursions between 15°C and 30°C are allowed and will not be considered deviations.

4.2 Acquisition/Distribution

The Norovirus GII.4 CIN-3 Batch No.: 01-16C3 will be received by CCHMC from Dr. Ming Tan at Cincinnati Children's Hospital Medical Center (Tan Laboratory).

Diluent - Sterile Water for Injection, USP - The sterile water for injection, will be provided by CCHMC. CCHMC will ship the sterile water for injection, USP, to CMS, Fisher BioServices.

Buffer - Sodium Bicarbonate, USP – The sodium bicarbonate for buffer will be provided by CCHMC. CCHMC will ship the sodium bicarbonate, USP, to CMS, Fisher BioServices.

4.3 Protocol-Specified Medications/Treatments other than Study Products

Subjects who develop diarrhea will be asked to drink 1.5 mL of ORS for each gram of diarrheal stool that they produce. Subjects developing vomiting will be asked to drink a volume of ORS equivalent to the amount of the emesis. Subjects unable to drink sufficient ORS to maintain their hydration status may be provided intravenous fluids to prevent and treat dehydration. Therapies including intravenous fluids and other medications (excluding ORS) will be recorded as concomitant medications. Subjects will be allowed to continue eating solid foods if they think they are well enough to tolerate a meal.

At the investigator's discretion and at the subject's request, subjects may be prescribed acetaminophen (or equivalent) for fever, headache, muscle aches, abdominal pain or other

symptoms. Other medications may be prescribed if the study physician feels the subject is at high risk for volume depletion.

Anti-emetic medication may be administered by PI discretion only. As this would potentially decrease the Vesikari score, anti-emetics only will be used if the subject has reached a high severity of illness and is at risk of requiring intravenous therapy if the emesis is not controlled. Ondansetron - (Orange Book #020781, Zofran® Oral Disintegrating Tablet) or its licensed equivalent may be administered as prescribed by the investigator. See the package insert for further dosing instructions. If the subject is unable to tolerate fluids by mouth and intravenous fluids are deemed necessary by the assessing investigator, a 0.9% sterile saline solution 1-liter bolus will be administered intravenously. Based on additional assessments, replacement intravenous fluids may be continued at investigator discretion.

4.4 Dosage/Regimen, Preparation, Dispensing and Administration of Study Intervention/Investigational Product

Refer to protocol-specific Manual of Procedures (MOP) for detailed instructions on the preparation, dispensing, and administration of the study product.

Investigational agents: The agent used in this subject challenge trial is a safety tested Norovirus GII.4 CIN-3 Batch No.: 01-16C3 inoculum (IND number pending).

Route and Frequency of Administration: Subjects will receive a single oral dose of Norovirus GII.4 CIN-3 Batch No.: 01-16C3 on Day 1 of their inpatient stay at the clinical site.

Norovirus dosage: the initial cohort will be receiving $3.5x10^3$ copies of norovirus. This dose was chosen as the starting dose because from a previous GII.4 NoV challenge study at CCHMC, approximately the same dose resulted in a 70% rate of infection and 50% rate of disease.

Dosage for subsequent cohorts will depend on the results of the first cohort, per Figure 1.

Preparation of dose: On the day of Norovirus challenge, the study product will be removed from the freezer and thawed on wet ice for approximately 30 minutes. The undiluted (stock), which contains approximately $3.5 x10^5$ copies/mL of the challenge virus, will be diluted further with sterile water for injection, USP to a volume sufficient in order to have the **desired challenge dose** contained in 1 mL. Please refer to the MOP for specific dilution instructions.

One milliliter of the **desired challenge strain dose** will be added to 80 mL of sterile water for injection, USP in preparation for administration to the study subject. This mixture is termed the final study product use material.

Prior to administration to the subjects, an aliquot of the final dilution of the challenge strain will be placed in a freezer tube, labeled according to contents, and kept on wet ice for no longer than 8 hours until stored frozen at ≤60^0C (see MOP specifications). This aliquot will be used to confirm the dose of norovirus administered to the subjects. Any remaining portion of the thawed aliquot that is not used the day the sample is thawed will be discarded.

The **final subject use material** (cup) is labeled with the patient's initials and study ID, the time/date of final preparation, an expiration time and date, and the statement "CAUTION-FEDERAL LAW PROHIBITS THE USE OF THIS PRODUCT EXCEPT FOR INVESTIGATIONAL USE". The final patient use material is stored short term at either 2-8ºC or held on wet ice for no more than 8 hours.

Administration: Once prepared, the challenge dose must be given within 30 minutes. On the day of challenge, subjects may have clear liquids within 4 hours of challenge but will be NPO (nothing by mouth) for at least 90 minutes prior to ingestion of 60 mL of a 2% sodium bicarbonate solution by mouth. Approximately 2 minutes later, subjects will be administered the **final patient use material.** Approximately five minutes following administration of the challenge dose, subjects will be administered a 60 mL volume of a 2% sodium bicarbonate solution and then remain NPO for at least the next 90 minutes. Subjects will be observed closely during the 60 minutes after receipt of the challenge by a study team member to detect and treat any immediate adverse reactions.

4.5 Pre-determined Modification of Study Intervention/Investigational Product for an Individual Subject

If a subject vomits the sodium bicarbonate solution or challenge dose, the dose will not be repeated.

4.6 Accountability Procedures for the Study Intervention/Investigational Product(s)

Once received, the challenge material will be stored in and dispensed by the Investigational Pharmacy.

The Food and Drug Administration (FDA) requires accounting for the disposition of all investigational products. The Investigator is responsible for ensuring that a current record of product distribution and disposition is maintained, and product is dispensed only at an official study site by authorized personnel as required by applicable regulations and guidelines. Records

of product disposition, as required by federal law, consist of the date received, date administered, quantity administered, and the subject number to whom the drug was administered.

The Investigational Pharmacist will be responsible for maintaining accurate records of the shipment and dispensing of the investigational product. The pharmacy records must be available for inspection by the DMID monitoring contractors and is subject to inspection by a regulatory agency (e.g., FDA) at any time. An assigned Study Monitor will review the pharmacy records.

Vials will be stored at $\leq 60^0$C until thawed and used. Used and unused vials of challenge strain and an aliquot of the final dilution of the challenge strain will be stored at ≤-60°C in a temperature controlled and continuously temperature monitored freezer in the Investigational Pharmacy until clinical trial accountability is completed and DMID provides instructions for disposition of the unused vials. At study termination, all unused investigational product will be handled in accordance with the MOP following complete drug accountability and monitoring.

5 SELECTION OF SUBJECTS AND STUDY ENROLLMENT AND WITHDRAWAL

5.1 Eligibility Criteria

Subjects must meet all of the inclusion criteria and none of the exclusion criteria to be considered eligible for enrollment. Subject Inclusion and Exclusion Criteria must be confirmed by a study clinician licensed to make medical diagnoses. No exemptions are granted on Subject Inclusion/Exclusion Criteria in DMID-sponsored studies. Questions about eligibility will be directed toward the DMID Medical Officer.

5.1.1 Subject Inclusion Criteria

1. Subject able to provide informed consent.
2. Male or non-pregnant females between the ages of 18 and 49 years, inclusive.
3. Women of childbearing potential must be using an acceptable method of birth control for at least 30 days prior to enrollment through day 45 after receipt of challenge virus.
   - A woman is considered of childbearing potential unless post-menopausal (absence of menses for ≥1 year) or surgically sterilized (tubal ligation, bilateral oophorectomy or hysterectomy).
   - Acceptable contraception methods for women include but are not limited to: sexual abstinence from intercourse with men, monogamous relationship with vasectomized partner who has been vasectomized for 6 months or more prior to the subject enrolling in the study, barrier methods such as condoms or diaphragms with spermicide or foam, effective devices (IUDs, NuvaRing®) or licensed hormonal products such as implants, injectables or oral contraceptives.
4. For women of childbearing potential, must have a negative serum or urine pregnancy test at screening.
5. Are in good general health*.

   * *As determined by medical history and physical examination to evaluate acute or currently ongoing chronic medical diagnoses or conditions, defined as those that have been present for at least 90 days, which would affect the assessment of the safety of subjects. Chronic medical diagnoses or conditions should be stable for the last 60 days (no hospitalizations, Emergency Room (ER), or urgent care for condition and no adverse symptoms that need medical intervention such as medication change/supplemental oxygen). This includes no change in chronic prescription medication, dose, or frequency as a result of deterioration of the chronic medical diagnosis or condition in the 60 days prior to enrollment. Any prescription change that*

is due to change of health care provider, insurance company, etc., or that is done for financial reasons, as long as in the same class of medication, will not be considered a deviation of this inclusion criterion. Any change in prescription medication due to ***improvement*** *of a disease outcome, as determined by the site principal investigator or appropriate sub-investigator, will not be considered a deviation of this inclusion criterion. Subjects may be on chronic or as needed (prn) medications if, in the opinion of the site principal investigator or appropriate sub-investigator, they pose no additional risk to subject safety or assessment of reactogenicity and immunogenicity and do not indicate a worsening of medical diagnosis or condition. Similarly, medication changes subsequent to enrollment and study vaccination are acceptable provided there was no deterioration in the subject's chronic medical condition that necessitated a medication change, and there is no additional risk to the subject or interference with the evaluation of responses to study vaccination.*

6. Demonstrate knowledge and comprehension of the study by scoring ≥70% on a quiz (test of understanding) of the study protocol and policies.
7. Willing and able to participate in all study visits, including an inpatient stay of at least 96 hours.

5.1.2 Subject Exclusion Criteria

Subjects who meet any of the following exclusion criteria will be excluded from study participation:

1. Have household contact with or have daily contact with children less than 2 years of age or persons older than 70 years of age.
2. Have expected extended social contact (> 2 hours/day) with immunocompromised individuals in the 8 weeks after challenge*.
   * *Including persons with HIV infection or active cancer, children <2 years of age, pregnant women or persons who are immunosuppressed (e.g. history of stem cell or organ transplantation) and/or provide any child day care services (in-home or non-residential facility).*
3. Are healthcare workers with direct patient contact or any child day care services (in-home or non-residential facility) in the 8 weeks after challenge.
4. Are positive for COVID-19 by an antigen test at the time of admission to the challenge unit.
5. Are food service workers expected to prepare/handle food in the 8 weeks after challenge.
6. Plan to be living in a confined communal environment (e.g. ship, camp, or dormitory) within 8 weeks after receiving the challenge strain.

7. For females, are pregnant or plan to become pregnant at any time between the Screening Visit through 45 days after receipt of the challenge virus.
8. Are breastfeeding or plan to breastfeed at any given time throughout the study.
9. Have a history of acute gastroenteritis in the 4 weeks prior to challenge or any history of chronic or recurrent diarrhea or vomiting.
10. History of significant GI condition.*
    **Including: malabsorption, major GI surgery, current *eating disorder, irritable bowel syndrome, or any GI disorder (deemed clinically significant by study physician) making it unsafe to participate.*
11. Have significant acute illness or an oral temperature ≥100.4°F within seven days prior to challenge.
12. Have a heart rate <45 beats per minute (bpm) or >100 bpm*.
    ** If heart rate is <45 beats per minute and the investigator determines that this is not clinically significant (e.g., athletes) and heart rate increases >45 beats per minute on moderate exercise (two flights of stairs), subject will not be excluded. If a subject has significant abnormalities in their heart rate, they will be informed of the values and advised to seek care from their physician.*
13. Systolic blood pressure less than 90 mm Hg or greater than 150 mm Hg on two separate measurements (screening and baseline prior to challenge).
    ** If a subject has significant abnormalities in their blood pressure, they will be informed of the values and advised to seek care from their physician.*
14. Diastolic blood pressure less than 50 mm Hg or greater than 90 mmHg on two separate measurements (screening and baseline, prior to challenge).
15. Have long-term use (≥2 weeks) of high-dose oral (≥20 mg per day prednisone or equivalent) or parenteral glucocorticoids, or high-dose inhaled steroids for greater than 7 days in the last 6 months.
16. Have an autoimmune, inflammatory, vasculitic or rheumatic disease, including but not limited to systemic lupus erythematosus, polymyalgia rheumatica, rheumatoid arthritis or scleroderma.
17. Have HIV, Hepatitis B, or Hepatitis C*.
    ** Subjects will be tested for HIV, Hepatitis B surface antigen, and antibody to Hepatitis C at screening only. Any subjects having HIV, Hepatitis B, Hepatitis C infection will not be enrolled into the study.*
18. Have a seizure disorder.
19. Have an active malignancy or history of malignancy* or current use of immunosuppressive or cytotoxic therapy.
    **Excluding nonmelanotic skin cancer in remission without treatment for more than 5 years*

20. Have abnormal screening laboratory test results per laboratory reported normal values and Appendix B*.
    * *For white blood cells (WBCs), hemoglobin (Hgb), platelets, absolute neutrophil count (ANC), total bilirubin, potassium, sodium, and urine protein.*
21. Serum creatinine greater than a Grade 1 adverse event on enrollment.
22. Alanine aminotransferase (ALT), greater than Grade 1 adverse event on enrollment.
23. Have a chronic condition that the study physician feels would pose a threat to participating subjects*.
    * *Including, but not limited to solid organ or stem cell transplantation, diabetes, clinically significant history of immunosuppressive illness, gall bladder disease, heart disease, lung disease, pancreatic disease, renal disease or neurological disease.*
24. Have ongoing drug abuse/dependence (including alcohol), or a history of these issues within 5 years of enrollment.
25. Have a positive urine test for opiates.
26. Have any medical, psychiatric, occupational, or behavioral problems that make it unlikely for the subject to comply with the protocol as determined by the investigator.
27. Are unwilling to comply with study procedures including abstaining from smoking for the duration of the inpatient portion of the study.
28. Have participated in a previous NoV challenge study or NoV vaccine study.
29. Have received experimental products within 30 days before study entry or plan to receive experimental products at any time during the study.
30. Plans to enroll in another clinical trial that could interfere with safety assessment of the investigational product at any time during the study period*
    * *Including study interventions such as drugs, biologics or devices.*
31. Plan to donate blood during the course of the study.
32. Have received a live vaccine within 30 days before study entry or plan to receive a live vaccine prior to Day 30 of the study.
33. Have received, or plan to receive, an inactivated vaccine within 14 days of challenge to 14 days after challenge.
34. Received parenteral immunoglobulin or blood products within 3 months of challenge, or plan to receive parenteral immunoglobulin/blood products within 3 months after challenge.
35. Use of antibiotics within 7 days prior to entry into the inpatient facility.
36. Use of prescription and OTC medications containing acetaminophen, aspirin, ibuprofen, and other non-steroidal anti-inflammatory drugs within 48 hours prior to NoV challenge.
37. Regular use of laxatives or anti-motility agents.

38. Have a history of allergy to sodium bicarbonate.
39. Have had a recent norovirus infection or have ever had a norovirus vaccine.

5.2 Withdrawal from the Study, Discontinuation of Study Product, or Study Termination

5.2.1 Withdrawal from the Study or Discontinuation of the Study Product

Subjects may voluntarily withdraw their consent for study participation at any time without penalty or loss of benefits to which they are otherwise entitled.

An investigator may also withdraw a subject from receiving the study product for any reason. Follow-up safety evaluations will be conducted, if the subject agrees. If a subject withdraws or is withdrawn prior to completion of the study, the reason for this decision must be recorded in the case report forms (CRFs).

The reasons, might include, but are not limited to the following:

- Prior to dosing, the subject no longer meets eligibility criteria
- Prior to dosing, the subject meets individual halting criteria
- Subject becomes noncompliant
- Medical disease or condition, or new clinical finding(s) for which continued participation, in the opinion of the investigator might compromise the safety of the subject, interfere with the subject's successful completion of this study, or interfere with the evaluation of responses
- Positive for COVID-19 by rapid antigen testing at the time of admission to the challenge unit
- Subject lost to follow-up
- Subject becomes pregnant
- Determined by a physician’s discretion to require additional therapy not indicated in the protocol to ensure subject’s health and well-being (or treatment failure, if applicable)

Whenever possible, subjects should continue to be followed for safety. The investigator should be explicit regarding study follow-up (e.g. safety follow-up) that might be carried out despite the fact the subject will not receive further study product. If the subject consents, every attempt will be made to follow all AEs through resolution. The procedures that collect safety data for the purposes of research must be inclusive in the original informed consent or the investigator may seek subsequent informed consent using an IRB/IEC-approved consent form with the revised procedures.

The investigator will inform the subject that already collected data will be retained and analyzed even if the subject withdraws from this study.

5.2.2 Subject Replacement

Subjects who withdraw, or are withdrawn from this study, or are lost to follow-up after signing the informed consent form (ICF) and administration of the study product will not be replaced. Subjects who withdraw, or are withdrawn from this study, or are lost to follow-up after signing the ICF but before administration of the study product may be replaced. At the time of admission to the inpatient unit, 2-3 additional subjects will be admitted and serve as alternates. The alternates would become primary study subjects if a subject in the primary group is unavailable or becomes ineligible at the time of the inpatient study. If alternates are not needed, they will be sent home before challenge and offered to be a primary subject in the next cohort. Alternate subjects will be re-screened if their screening information falls outside the protocol designated window for a subsequent cohort.

5.2.3 Study Termination

If the study is prematurely terminated by the sponsor, any regulatory authority, or the investigator for any reason, the investigator will promptly inform the study subjects and assure appropriate therapy or follow-up for the subjects, as necessary. The investigator will provide a detailed written explanation of the termination to the IRB/IEC. If any subject's private information will continue to be collected for this study, the IRB/IEC must approve a consent form with the study procedures, any risks and discomforts, and applicable elements, and the investigator or designee will re-consent the subjects as approved by the IRB/IEC.

6 STUDY PROCEDURES

6.1 Screening Visit 00A (Day -60 to -2)

After signing the informed consent form, subjects will be carefully screened with a detailed medical history and physical examination to ensure they are in good physical and mental health. Screening may be completed at one or more visits depending on the subject and the timing of specimen collection.

Screening evaluations will include the following and may occur over 1 or more visits as outlined below:

An initial screening visit may be scheduled to obtain informed consent, provide educational materials for the test of understanding, review inclusion/exclusion criteria, obtain demographics, measure body weight and vital signs (including oral temperature, blood pressure, and pulse), and collect medical history (including concomitant medications and allergies).

Specimens will be collected for hematology testing (hemoglobin, white blood cells with neutrophil count and platelet count), chemistry panel (sodium, potassium, creatinine, ALT, total bilirubin), urine or serum pregnancy testing (for females of childbearing potential), serology including Hepatitis B surface antigen, antibody to Hepatitis C, HIV antibody, and urine collection for protein and opiates. Saliva will be collected and tested for secretor status. Repeat testing may be completed at a subsequent visit.

Subjects will be scheduled to have an abbreviated physical (genital-urinary and rectal exam not required).

A test of understanding will be administered prior to challenge. Subjects must pass with a score of at least 70%. Anyone not passing the test initially may retake the test with a passing score.

Screening must take place between 60 and 2 days before enrollment (day of challenge). Subjects whose date of screening falls outside of the screening period must be re-screened before they may be enrolled.

Subjects will be given a stool kit to bring a baseline sample to the inpatient challenge.

If a physiologic parameter, e.g., vital signs, is outside of the protocol-specified range, then the measurement may be repeated once if, in the judgment of the investigator, the abnormality is the result of an acute, short-term, rapidly reversible condition (e.g., stress, anxiety, or “white coat

syndrome"). A physiologic parameter may also be repeated if there is a technical problem with the measurement caused by malfunctioning, or an inappropriate measuring device (i.e., inappropriate-sized blood pressure cuff).

If hemoglobin, WBC, platelet or neutrophil count, blood chemistry, or urine protein is out of range, then the measurement may be repeated once if there is a medical reason to explain the values being out of range. If the repeat value remains out of range but determined by the study investigator not to be clinically significant, the subject may continue. For baseline laboratory results that are abnormal according to the local laboratory reference range and fall within Grade 1 toxicity table range, these will not be considered exclusionary if determined by the investigator to be not clinically significant. Laboratory values that meet the post dose grading criteria but did not preclude the subject from enrolling will be entered as AEs only if the value worsens in severity. These values will be considered a pre-existing condition and documented on the Medical History.

A subject may be re-screened if there is a transient disease status (e.g., subject complained of a "cold or fever" and met a temporary delaying enrollment criterion of acute illness or fever). A subject may also be re-screened if the subject was screened eligible for a previous dose group but was not enrolled (e.g., subject was an alternate, was unable to produce a baseline stool sample, has scheduling conflict with inpatient admission days, etc.).

If screening labs are collected outside of the designated window of -60 and -2 days before challenge, the screening labs may be re-collected to determine if the subject remains eligible for study participation.

No subject may be screened more than twice due to a screening failure result as defined above. Repeating of a lab value if the original result is out of window is not considered re-screening for screen failure. Once the screening evaluation is complete, eligibility for continuation to the inpatient challenge portion will be determined. Those who remain eligible and are willing to participate will be scheduled for admission to receive the challenge dose. Inpatient Stay (Admission Day to post challenge Day 5)

6.1.1 Screening Visit 00B (Day -1)

Staff will confirm that subjects have completed informed consent and will be asked to read and sign any updated consent document(s).

Subjects who have not previously taken the test of understanding will take the test and must pass with a score of at least 70%.

Demographics will be confirmed. Body weight and vital signs (including oral temperature, blood pressure, and pulse) will be obtained, and a review of medical history (including concomitant medications and allergies) will be completed. A symptom directed physical exam will be performed, females of child-bearing potential will have a confirmed negative urine pregnancy test, a COVID-19 antigen test will be performed and confirmed as negative, and review of inclusion/exclusion criteria will be performed to ensure subject eligibility.

Blood and stool samples will be collected per schedule of events.

6.1.2 Inpatient Challenge Admission

Subjects either may be admitted the day before challenge or on the day of challenge.

The study will be conducted in the Cincinnati Children's Hospital Medical Center (CCHMC) inpatient unit. The unit has controlled access and is comprised of up to 20 rooms that can be utilized as private or semi-private rooms, each with its own private bathroom.

During the stay in the inpatient facility, visitors will not be permitted, and subjects will not be allowed to leave the inpatient facility until they have been cleared by a member of the study team. No other clinical trials will be conducted in the inpatient facility during the time this study is being conducted.

Meals and any medications needed to treat clinical symptoms associated with norovirus illness will be provided for the study subjects during their stay in the inpatient facility. Medicines routinely taken by subjects may be taken per their usual routine, if approved by investigators. While subjects are in the inpatient unit, nurses and other clinical staff will be on the unit continuously to provide medical supervision. A physician or delegate will be on-call during the entire inpatient period and will be available to assess subjects at any time deemed necessary by the nursing staff.

Antigen test for COVID-19

Any participant who tests positive for COVID-19 will be immediately escorted out of the challenge unit and will have no further participation in that cohort.

Review medical history including concomitant medications to ensure still meet eligibility criteria

Review inclusion/exclusion criteria

Symptom directed physical examination

Vital signs including oral temperature, blood pressure, and pulse.

Baseline weight

Serum or urine pregnancy test for women of childbearing potential (May be done up to 24 hours prior to challenge).

Obtain baseline specimens per Schedule of Events Appendix A.

Blood will be collected for:

- norovirus GII.4-specific IgG, IgA and Blocking Antibody, ELISA
- norovirus GII.4-specific Memory B cells by ELISpot assay
- norovirus GII.4-specific ASC ELISpot and ALS by ELISA
- Future Use

Stool will be collected for:

- Virus Detection
- Future Use

Challenge Procedure (Day 1)

Subjects will not be allowed to eat or drink anything for at least 90 minutes before the Challenge Procedure begins.

First, subjects will be administered 60 mL of a 2% sodium bicarbonate solution by mouth.

Approximately two minutes later, administer the GII.4 norovirus Challenge Dose by mouth. (Challenge dose will be prepared as outlined in the Manual of Procedures)

Approximately five minutes following administration of the challenge dose, subjects will be administered a 60 mL volume of a 2% sodium bicarbonate solution. Subjects will be observed closely during the 60 minutes after receipt of the challenge by a study team member to detect and treat any immediate adverse reactions. Vital signs will be taken at approximately 30 and 60 minutes post challenge.

Subjects may not take anything by mouth for 90 minutes after dosing

Within 12 hours of challenge, a physician or delegate will perform a symptom directed physical examination.

6.1.3 Inpatient Post-Challenge: Visit 02-05 (Days 2-5)

For the remainder of the inpatient period, the following procedures will be performed:

Vital signs (pulse, blood pressure and oral temperature) will be measured at least daily, more often as clinically indicated.

Weight will be obtained if there are concerns for dehydration.

Symptom directed examinations will be performed by physician or delegate at least daily, more often as clinically indicated.

Assessment of solicited AEs (symptoms of fever, headache, nausea, abdominal cramps/discomfort/pain, abdominal gurgling abdominal bloating, myalgia, malaise/fatigue, anorexia/loss of appetite, chills) will be completed at least once daily until discharge.

Record number of episodes of vomiting and diarrhea.

All stools will be graded for consistency (see Appendix D: Diarrhea/Vomiting Grading Scale for details), and all loose or watery stools (i.e., those that conform to the shape of the container) will be weighed.

The first stool of each study day will be collected (study day is defined as 00:00 – 23:59) for viral detection.

Subjects with diarrhea will have up to 1 stool sample/8 hours collected.

Input and output will be monitored for any subject with vomiting and/or diarrhea.

Evaluation for dehydration. A physician, APN or PA will evaluate subjects who are symptomatic and have any of the following:

- Tachycardia (resting heart rate >100 beats/min)
- Hypotension (systolic pressure <90 mm Hg or diastolic pressure <50 mm Hg accompanied by symptoms of orthostasis)
- Greater than 1000 mL deficit in ORS replacement in 24 hours

If deemed clinically necessary by the physician or delegate, urine testing for specific gravity may be done.

Review of AEs and SAEs will be completed.

Record any medications taken.

Collect study samples as described in Schedule of Events Appendix A and listed below:

Blood will be collected for:

- Future Use Day 2 and Day 4
- Safety Labs (hematology and chemistry) Day 5 if indicated per investigator discretion, prior to discharge

Stool will be collected for:

- Virus detection Day 2, Day 3, Day 4, Day 5
- Future Use Day 2, Day 4

Treatment of vomiting and/or diarrhea

See section 4.3 for allowed treatment of vomiting and diarrhea.

Subjects will remain in the inpatient facility for a minimum of four days following challenge. Subjects will be discharged from the inpatient facility when the following criteria are met:

- At least 96 hours after administration of the norovirus challenge
- Afebrile for at least the 12 hours prior to discharge
- Able to maintain hydration
- Clinically stable
- If having any symptoms, they do not interfere with activities of daily living
- Received education prior to discharge about the potential for transmission of norovirus to others up to 30 days after leaving the challenge unit

- Received instructions to contact the site if signs or symptoms of illness develop after discharge

Prior to discharge, all subjects will have the following performed:

Obtain blood for safety labs, if indicated per investigator discretion, prior to discharge

Subjects who receive IV hydration during the inpatient admission will be required to have clinically acceptable electrolyte values for sodium and potassium prior to discharge.

Provide subject with a digital thermometer and instructions in its use and asked to take and record their oral temperature once daily at the same time each day for 5 days after discharge. Subjects should record their highest temperature taken each day, if additional temperatures are measured by the subject for fever

Provide subject with an illness/reactogenicity (solicited adverse events) memory aid and instruct them to record information for 5 days after discharge

Instruct subject to report any unsolicited adverse events through study Day 30 after study product administration

Subjects will be instructed to contact the site if a household contact or anyone they have daily contact with develops symptoms of Norovirus infection within a week after being discharged from the inpatient unit.

Instruct the subject to notify the study staff promptly if they develop any signs or symptoms of illness, become pregnant, or require hospitalization during the 30-day period after challenge

Instructions on when to return to the outpatient clinic for evaluation

Provided a stool collection system ("stool kit") and asked to bring a stool sample to the outpatient clinic visit

6.2 Outpatient Post Challenge Visits

6.2.1 Visit 06, Day 6 (+2, Day 6-8)

At the Day 6 visit, the following procedures will be performed:

Review interim medical history and record concomitant medications

Perform a symptom-directed physical exam if indicated

Collect vital signs including oral temperature, blood pressure, and pulse

Blood will be collected for:

- norovirus GII.4-specific ASC ELISpot and ALS ELISA
- Future Use

Stool will be collected for:

- Virus detection
- Future Use

If the subject was unable to provide a bulk stool sample from home, an attempt will be made to have the subject collect a bulk stool during the clinic visit.

Review the subject's illness/reactogenicity (solicited adverse events) memory aid.

Remind subject to continue recording illness/reactogenicity (solicited adverse events) information until Day 10 and to bring the memory aid to the next clinic visit.

Remind subject to report any unsolicited adverse event through study Day 30 and to notify the study staff promptly if they develop any signs or symptoms of illness, become pregnant, or require hospitalization during the 30-day period after challenge.

Remind subjects to contact the site if a household contact or anyone they have daily contact with develops symptoms of Norovirus infection within a week after being discharged from the inpatient unit.

Review of AEs and SAEs

A stool kit will be given to the subjects and they will be asked to bring a stool sample with them for the next study visit.

6.2.2 Visit 07, Day 15 (+/-1, Day 14-16)

The following procedures will be performed at this visit:

Review interim medical history and record concomitant medications

Perform a symptom-directed physical exam (obtain vital signs, if indicated)

Blood will be collected for:

- Serum norovirus IgG (including blocking antibody), and serum IgA ELISA
- norovirus GII.4- specific ASC ELISpot and ALS ELISA
- Future Use

Stool will be collected for:

- Virus detection
- Future Use

If the subject was unable to provide a bulk stool sample from home, an attempt will be made to have the subject collect a bulk stool during the clinic visit.

Review subject's illness/reactogenicity (solicited adverse events) memory aid and collect.

Remind subject to report any unsolicited adverse events through Day 30 after study product administration.

Remind the subject to notify the study staff promptly if they develop any signs or symptoms of illness, become pregnant, or require hospitalization during the 30-day period after challenge

Review of AEs and SAEs

Ask subjects if a household contact or anyone they have daily contact with has developed symptoms of Norovirus infection within a week after being discharged from the inpatient unit.

A stool kit will be given to the subjects and they will be asked to bring a stool sample with them for the next study visit.

6.2.3 Visit 08, Day 30 (-2/+5, Day 28-35)

The following procedures will be performed at this visit:

Review interim medical history

Review and record concomitant medications

Collect unsolicited adverse events through study Day 30 after product administration

Perform a symptom-directed physical exam (obtain vital signs, if indicated)

Blood will be collected for:

- Serum norovirus IgG IgA ELISA and blocking antibody

- norovirus GII.4- specific Memory B-cells
- Future Use

Stool will be collected for:

- Virus detection
- Future Use

If the subject was unable to provide a bulk stool sample from home, an attempt will be made to have the subject collect a bulk stool during the clinic visit.

Review of AEs, Pregnancy, and SAEs

A stool kit will be given to the subjects and they will be asked to bring a stool sample with them for the next study visit

6.2.4 Visit 09, Day 45 (-5/+5, Day 40-50)

The following procedures will be performed at this visit:

Perform a symptom-directed physical exam (obtain vital signs, if indicated)

Review interim medical history

Stool will be collected for:

- Virus detection

Review of pregnancy, SAEs that have occurred since the last visit

A stool kit will be given to the subjects and they will be asked to bring a stool sample with them for the next study visit

6.2.5 Visit 10 Day 60 (-5/+5, Day 55-65)

Review interim medical history.

Review of pregnancy, SAEs that have occurred since the last visit

Perform a symptom-directed physical exam (obtain vital signs, if indicated)

Blood will be collected for:

- norovirus GII.4- specific Memory B-cells

Stool will be collected for:

- Virus detection
  - If the subject was unable to provide a bulk stool sample from home, an attempt will be made to have the subject collect a bulk stool during the clinic visit.

If shedding is detected by qRT-PCR in the Day 45 stool sample, the subject will be asked to complete a Day 60 visit to provide a stool sample for virus detection. Subjects who continue to shed Norovirus at the Day 60 visit will return every 2 weeks for testing until shedding no longer is detected. The visit procedures would be the same as listed above for Day 60 visit.

6.3 Final Study Contact

Final Study Contact: Visit 11, Day 180 (+/-14, Day 166-194)

Approximately 180 days after receiving virus challenge, a final study contact call will be performed to obtain an interim medical history. Review pregnancy, SAEs that have occurred since the last visit.

6.4 Early Inpatient Discharge

In the case of subjects who need to leave the inpatient facility early (before 4 days after receipt of the challenge) due to an emergency, the following activities will be performed:

- Subjects will be instructed in hand hygiene techniques to minimize the potential spread of norovirus to their family members and other close contacts.
- Subjects will be asked to undergo an evaluation by the investigator.
- Scheduled study labs will be collected.
- Review of AEs and SAEs.

Subjects will still be asked to complete the illness/reactogenicity (solicited adverse events) and unsolicited adverse event(s) memory aids, complete procedures indicated for that study day, including safety labs if indicated per investigator discretion, and complete all remaining study visits and final study contact.

6.5 Transfer to acute care hospital

If the subject needs to be transferred to a hospital for additional care during his/her inpatient facility stay, the study staff will ask permission of the subject to obtain additional medical records to follow the subject until the symptoms of this serious adverse event resolve or the subject's condition becomes stable. The hospital and physicians accepting the subject for care will be informed of the subject's participation in this research study so that the subject can be managed in appropriate isolation precautions.

6.6 Unscheduled Study Visits

Subjects will be asked to notify the study staff promptly if they develop any illness suggestive of recurrence of the norovirus (vomiting or diarrhea) or possible dehydration. If the study staff determines the symptoms are potentially significant, the subject will be asked to come to the clinic for an evaluation. Subjects will be asked to complete an unscheduled visit for any event that warrants follow-up. All events will be followed to resolution or until determined to be stable.

6.7 Protocol Deviations

A protocol deviation is any noncompliance with the clinical trial protocol, GCP, or protocol-specific MOP requirements. The noncompliance may be either on the part of the subject, the site principal investigator, or the site personnel. As a result of deviations, corrective actions are to be developed by the site and implemented promptly.

These practices are consistent with ICH E6:

4.5 Compliance with Protocol, Sections 4.5.1, 4.5.2, and 4.5.3

5.1 Quality Assurance and Quality Control, Section 5.1.1

5.20 Noncompliance, Sections 5.20.1, and 5.20.2.

It is the responsibility of the site principal investigator and personnel to use continuous vigilance to identify and report deviations within five working days of identification of the protocol deviation, or within five working days of the scheduled protocol-required activity. All deviations must be promptly reported to DMID per the DCC protocol deviation reporting procedures.

All protocol deviations, as defined above, must be addressed in study subject data collection forms. A completed copy of the DMID Protocol Deviation Form must be maintained in the

Regulatory File, as well as in the subject's chart. Protocol deviations must be sent to the local IRB/IEC per their guidelines. The site principal investigator and personnel are responsible for knowing and adhering to their IRB requirements.

7 DESCRIPTION OF CLINICAL AND LABORATORY EVALUATIONS

7.1 Clinical Evaluations

Medical History

Medical history will be obtained by direct interview and will include a review of concomitant medications, supplements, and over the counter (OTC) medications. Subjects will be queried regarding a history of significant medical disorders of the head, eyes, ears, nose, throat (HEENT), mouth, cardiovascular system, lungs, gastrointestinal tract, liver, pancreas, kidney, urologic, nervous system, blood, lymph glands, endocrine system, musculoskeletal system, skin, and genital/ reproductive tract. A history of any allergies, cancer, gastrointestinal disorders, immunodeficiency, psychiatric illness, substance abuse, and autoimmune disease will be solicited.

Physical Examination

An abbreviated physical examination will be conducted at the screening visit. An abbreviated physical examination is distinguished from a complete physical exam in that all assessments are not required (e.g. genito-urinary and rectal exams). A rectal examination may be performed at screening if, in the opinion of the investigator, this assessment is warranted based on the subject's medical history. A symptom directed physical examination may be performed at all other study visits if indicated based on, reactogenicity (solicited adverse events) or interim medical history review.

Inpatient Evaluations

During the Inpatient Stay, the following clinical evaluations will occur:

Subjects will be asked to collect every stool passed during the inpatient portion of the study.

Body weight will be measured on admission to the inpatient unit and repeated during the inpatient stay as clinically indicated to evaluate for dehydration.

Vital signs including oral temperature, blood pressure and pulse will be obtained at least daily, or more frequently as clinically indicated

Daily evaluations by an investigator, or more frequently as clinically indicated, to check for complaints of: fever, headache, nausea, abdominal cramps/discomfort/pain, abdominal gurgling, abdominal bloating, myalgia, malaise/fatigue, anorexia/loss of appetite, chills.

Clinical Laboratory Evaluations

Clinical laboratory evaluations of the subjects will include collection of blood, urine, saliva, and stool. Testing to be performed as outlined in Appendix A. Blood volumes are listed in Table 1.

Saliva and stool volumes are listed in Table 2.

Safety laboratory evaluations will be performed by the local lab of the performance site. Urine screening for opiates will be performed. Urine pregnancy testing and urine dipstick for protein will be performed in the clinic by a qualified study team member using a commercially available test.

Subjects will be tested for HIV, Hepatitis B surface antigen, and antibody to Hepatitis C at screening only. For females of childbearing potential, a urine or serum pregnancy test will be performed at screening. Urine or serum pregnancy testing will be performed within 24 hours of challenge administration. Pregnancy test results must be negative for subjects to be eligible for participation.

Saliva will be collected at screening for determination of secretor status.

Clinical safety lab testing will include hematology, chemistry and urinalysis with the following parameters.

Hematology: white blood cell count (WBC), absolute neutrophil count (ANC), hemoglobin (Hgb), and platelet count

Chemistry: sodium, potassium, creatinine, ALT, and total bilirubin

Urinalysis by dipstick for protein. At screening, a result greater than trace protein will make the subject ineligible for study participation. Any other parameters on the dipstick having a positive result are not exclusionary and the decision to enroll the subject is at the discretion of the investigator.

Table 1: Venipuncture/Blood Volumes (mL)

Study Day	Tube Type	Screening (-60 to-2)	Admission (Day -1)	1 (day of challenge)	2	3	4	5	6 (+2)	15 (± 1)	30 (-2, +5)	45 (±5)	60 (±5)	TOTAL
Chemistry and Hematology Safety Labs	**5mL EDTA, 5mL SST**	**10**		**10#**	**10#**	**10#**	**10#**	**10#**						**10**
HIV, HepC Ab, HepB surface Ag, pregnancy test* (if determined by serum)	**10 mL SST**	**10**												**10**
Serum Norovirus Antibody (IgG and IgA ELISA and blocking antibody)	**10 mL SST**		**10**	**(10)**						**10**	**10**			**30**
RNAseq assay Future Use	**2.5 mL PAXgene**		**2.5**	**(2.5)**	**2.5**		**2.5**		**2.5**	**2.5**	**2.5**			**15**
Protein (cytokine) Luminex assays (plasma from PBMC processing, no extra blood) Future Use**			**x**	**(x)**	**x**		**x**		**x**	**x**				**0**
DC/NK (PBMC) Future Use	**8.0 mL CPT**		**8**	**(8)**	**8**		**8**		**8**	**8**				**40**
norovirus GII.4-specific Memory B cells/ELISpot assay (PBMC)	**8.0 mL CPT**		**16**	**(16)**							**16**		16	**48**
norovirus GII.4—specific T cell/Intracellular cytokine assay (PBMC) Future Use	**8.0 mL CPT**		**8**	**(8)**					**8**	**8**	**8**			**32**
Norovirus GII.4—specific ASC ELISpot, ALS ELISA	**8.0 mL CPT**		**32**	**(32)**					**32**	**32**				**96**
Total Blood Volume		**20.0**	**76.5**		**10.5**		**10.5**		**50.5**	**60.5**	**36.5**		16	**281**

* volume may vary depending on the gender of the subject (i.e. serum pregnancy testing)

** plasma from PBMC processing, no extra blood
( ) these samples may be collected either on admission or Day 1, prior to challenge. These samples are collected **once** within 24 hours of challenge.
these samples may be collected at the discretion of the investigator

At the discretion of the investigator or delegate, urine dipstick may be performed to capture urine specific gravity results in real time.

Table 2. Saliva and Stool Collection

Study Day	**Screening** (-60 to-2)	**Admission** (Day -1)	**1** (day of challenge)	**2**	**3**	**4**	**5**	**6 (+2)**	**15 (± 1)**	**30 (-2, +5)**	**45 (±5)**	**60 (±5)**	**TOTAL**
Saliva sample	**X 5 mL**												**5 mL**
Stool samples		**X**	**(X)**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X****	

** Subjects who continue to shed Norovirus at the Day 60 visit will be followed for clearance

Research Procedures

Subjects will be provided with an illness/reactogenicity (solicited adverse events) memory aid and asked to record information regarding diarrhea and vomiting, and any solicited events (anticipated systemic reactions) for 5 days after discharge.

Subjects will be asked to report any unsolicited adverse events through study Day 30 after study product administration.

During the inpatient admission, symptom directed physical examinations will be performed daily, or more often as clinically indicated, by qualified medical staff. At the same time, the medical staff will gather information from the subject regarding complaints of fever, headache, nausea, abdominal cramps/discomfort/pain, abdominal gurgling, abdominal bloating, myalgia, malaise/fatigue, anorexia/loss of appetite, and chills.

Vomiting (number of times per day and weights) episodes will be recorded.

Diarrheal (as defined per protocol) stools will be weighed, recorded and graded for stool consistency. Stool will be collected for virus detection.

Assessment of Concomitant Medications/Treatments other than Study Product

Administration of any medications or vaccines will be documented in the appropriate eCRF. All concomitant medications, taken in the 30 days prior to study enrollment through Day 30 or early termination, whichever occurs first, will be recorded. All prescription and over-the-counter medications as well as vitamins and supplements will be recorded.

Assessment of eligibility also will include a review of permitted and prohibited medications (per the exclusion criteria).

Use of new medications should prompt evaluation for the presence of an adverse event.

Medications which may interfere with the evaluation of the investigational product should not be used unless absolutely necessary. Medications or treatments which are prohibited through study Day 30 include:

- Vaccines (inactivated vaccines, if clinically necessary, should be administered 14 days before or 14 days after challenge)
- Oral, parenteral or high-dose inhaled steroids
- Immunosuppressive or cytotoxic therapy
    - Blood products or immunoglobulins
    - Experimental products (through study Day 180)

Any medications considered for treatment of fever, adverse event, or reactogenicity (solicited adverse events) will be given only at the discretion of the study investigator.

Assessment of Subject Compliance with Study Intervention/Investigational Product/Investigational Device

Subjects are expected to drink all of the product mixture, as well as 60 mL of 2% sodium bicarbonate, approximately 2 minutes before and another 60 mL approximately 5 minutes after dose administration. Study staff will record in the source document the time the sodium bicarbonate solutions and the inoculum were administered. Subjects will be observed by study staff for compliance.

If a subject vomits the inoculum or the sodium bicarbonate solution, the dose will not be repeated. In this scenario, the subject would remain in the isolation unit and have specimens collected per protocol. The subject will remain in the study until symptoms resolve and he/she meets the discharge criteria and will continue to be followed for safety, if possible. If the subject

drinks part of the inoculum, then refuses to drink the rest of the inoculum or sodium bicarbonate solution, the subject would remain in the isolation unit and have specimens collected per protocol. The subject will remain in the study until symptoms resolve and he/she meets the discharge criteria, and will continue to be followed for safety, if possible. Subjects who refuse to drink any of the inoculum, and do not ingest any part of the inoculum, may be discharged immediately.

Subjects will be directly observed at the time of dosing by a member of the clinical research team who is licensed to administer the study product. Administration will be documented in the subject's source document and entered into the eCRF.

7.2 Laboratory Evaluations

Clinical Laboratory Evaluations

Safety laboratory evaluations will be performed by the local lab of the performance site.

Clinical safety lab testing will include hematology, chemistry and urinalysis. For baseline laboratory results that are abnormal according to the local laboratory reference range and fall within Grade 1 toxicity table range, these will not be considered exclusionary is determined by the investigator to be not clinically significant.

- Hematology: white blood cell count (WBC), absolute neutrophil count (ANC), hemoglobin (Hgb), and platelet count
- Chemistry: sodium, potassium, creatinine, ALT, and total bilirubin

A low creatinine value, a low total bilirubin value, and/or a low ALT value are/is acceptable for study inclusion. No lower limits of lab values for creatinine, total bilirubin, and ALT are considered to be clinically significant.

Urinalysis by dipstick for protein. At screening, a result greater than trace protein will make the subject ineligible for study participation. Any other parameters on the dipstick having a positive result are not exclusionary and the decision to enroll the subject is at the discretion of the investigator.

Urine screening for opiates will be performed. Any subject testing positive for urine opiates will not be eligible for study enrollment.

Urine pregnancy testing and urine dipstick will be performed in the clinic by a qualified study team member using a commercially available test.

For females of childbearing potential, a urine or serum pregnancy test will be performed at screening. Urine or serum pregnancy testing will be performed within 24 hours of challenge administration. Pregnancy test results must be negative for subjects to be eligible for participation.

Subjects will be tested for HIV, Hepatitis B surface antigen, and antibody to Hepatitis C at screening only. Any subjects having HIV, Hepatitis B, or Hepatitis C infection will not be enrolled into the study.

A commercially available COVID-19 antigen test will be performed on all participants upon entry into the challenge unit. Anyone with a positive test will be immediately escorted out of the challenge unit.

Research Assays

Special assays described below will be performed in the research laboratories of the performance site. The details of type of samples and sample collection time are described in the Appendix A. Blood volumes for the study are listed in Table 1: Venipuncture/Blood Volumes (mL).

Phenotypic

The secretor status of individual subjects will be determined based on the detection of histo-blood group antigens (HBGAs) for the secretor antigens (Le b, Le y, H types) vs. the non-secretor antigens (Le a and Le x) in their saliva. In this assay, boiled human saliva samples are diluted and used to coat wells of a 96-well EIA (Enzyme Immunosorbent Assay) plate in duplicate. The HBGAs present in a sample are determined by testing with a panel of monoclonal antibodies specific to each individual HBGA. A set of saliva samples with known HBGA profiles are used as positive and negative controls, including at least one positive and one negative control sample for each assay. An OD result >0.2 obtained after application of horseradish peroxidase (HRP) conjugated anti-mouse antibody of the correct isotope determines if a sample is positive for a particular HBGA.

Detection of Serum Norovirus IgG and IgA by Direct Capture ELISA

96-well microtiter plates are coated with rabbit antibody specific to noroviruses for overnight at 4ºC. Following a wash, the plates are blocked with 5% nonfat dry milk. Purified GII.4 Norovirus P-particles (PP) are then added. After incubation 1 hour at 37ºC, subject serum samples are added and the plates are incubated at 37ºC for 1 hour. HRP conjugated goat anti-human IgG antiserum is then added to each plate. After incubation the wells are washed and TMB substrate

solution is added to each well followed by incubation at room temperature. The reaction is stopped by the addition of 1M Phosphoric Acid. Plates are read using an EIA spectra reader (at a wavelength of 450 nm according to manufacturer's instructions). All assays will include positive and negative control serum.

Detection of Serum Norovirus Blocking Antibody by Receptor Blocking Assay

The ability of antibodies to blocking norovirus binding to HBGA receptors will be studied as a potential "neutralization" test by in vitro blocking assay on the norovirus P-particle binding to variable HBGAs. Briefly, saliva samples from individuals with known HBGA types or oligosaccharides representing different HBGAs will be coated on microtiter plates. The test antibodies (subject serum samples) will be incubated with P-particles in separate plates at 37°C for one hour before being transferred to the test plates. The bound P-particles will be measured by a guinea pig antibody specific to noroviruses followed by an HRP-conjugated goat anti-guinea pig antibody following the same procedures described above. The highest dilution of the testing antibodies with 50% or 90% reduction of binding signals in comparison with controls without blocking will be used as the blocking titer for each sample.

qRT-PCR (Real-time RT-PCR) for Quantitation of Norovirus RNA in stool

This method will be the primary method for norovirus detection in the stool. Stool suspensions (10% w/v) will be prepared in sterile water. Viral RNA will be purified from the clarified extracts using the QiaAmp Viral Mini kit (Qiagen) according to the manufacturer's instructions. The viral RNA will be eluted in a final volume of 60ul. Each sample will be tested using the TaqMan Fast Virus 1-Step Master Mix (Applied Biosystems) with forward primer – QNIF2d 5'ATG TTC AGR TGG ATG AGR TTC TCW GA, reverse primer – Cog2R 5'TCG ACG CCA TCT TCA TTC ACA, and probe RING2 5'6-FAM-TGG GAG GGC GAT CGC AAT CT-MGBNFQ. Real-time RT-PCR will be performed using the 7500 Fast Real-time PCR System (Applied Biosystems). Genome copies per gram of stool will be determined based on a standard Norovirus GII.4.

Norovirus GII.4- specific *Memory B Assay*- The memory B cell assay will be performed on frozen PBMC cells. PBMCs will be placed under in vitro polyclonal stimulation with mitogen for 6 days prior to addition to norovirus GII.4 P-particle coated plates (CIN-3; Virus Batch #01-16C3). Bound antibodies will be detected by the sequential addition of a biotinylated anti-Ig antibody followed by enzyme-conjugated Streptavidin. The assay is developed by the addition of a precipitating substrate and the spots can be enumerated with an ELISpot Reader. The number of memory B cells per 10^6 PBMC will be reported.

Antibody Secreting Cells (ASC) and Antibody in Lymphocyte Supernatant (ALS)

At times specified in the protocol and the MOP, the peripheral blood mononuclear cells (PBMCs) will be isolated from whole blood and cryopreserved to be used in ASC and ALS assays. An ELISPOT will be used to enumerate Norovirus CIN-3 specific IgA and IgG ASCs per total IgA and IgG ASCs. The ALS assay will measure by ELISA the secretion of Norovirus-specific antibodies in cultures of PBMCs.

Future Studies

Stool and blood, specimens will be preserved for future use. Details of assays, methodology and location of testing will be determined at a later date.

Additional innate and adaptive immunity assays: RNA, Protein (cytokine), and Cellular assays

RNA assay: Whole blood will be collected into PAXgene tubes. Samples will be remained at room temperature for 2 hours, moved to – 20 °C for 2h hours, and then transferred to a temperature controlled and continuously monitored freezer at ≤-60 °C for longer-term storage. Total RNA will be then extracted and used for RNA-seq analysis to identify molecular signatures either at baseline or induced early after norovirus challenge that correlate with and predict norovirus illness, infection, and/or adaptive immune responses.

Protein (Cytokine) assay: Plasma will be saved at PBMC processing for inflammation markers/ Luminex assay using commercially available kits. Depending upon the results from the RNA-seq analysis, various cytokines may be tested or obtained from commercially available sources.

Cellular Assays: PBMCs will be collected, processed and stored at CCHMC in liquid N_2 vapor phase until used for phenotyping of antigen presenting cells (APC) and NK cells. Further assays details are as described below:

Assay for frequency and phenotype of APCs. A panel of BDCA-1+ myeloid dendritic cells (mDC), cross-presenting CD141+ mDC, plasmacytoid DCs (pDC) as well as the pro-inflammatory subset of CD14+CD16+ monocytes will be analyzed. Subsets of APCs and monocytes for activation phenotype using the CD80, CD83, CD86 and CCR7 surface markers will also be detected.

Assay for frequency and activation status of NK cells: CD16+CD56+ NK cells and CD4+ and CD8+ T cells will be assessed by CD69 and HLA-DR and CD38 surface marker staining.

Mucosal homing: α4β7 will be detected on the surfaces of challenge-induced P-particle B cells and P-particle T cells.

CIN-3; Virus Batch #01-16C3-specific and cross reactive T cell response will be assessed by intracellular cytokine staining (ICS) analyzed with multicolor flow cytometry. PBMCs will be activated ex vivo with P-particles and brefeldin A (a protein transport inhibitor) will be added to retain the cytokines within the cells. After staining of cell surface with anti CD3/CD4/CD8 antibodies, cells are then fixed in paraformaldehyde and permeabilized. A panel of anti- cytokine antibodies (IFNg/IL2/IL13/IL17/IL21) will then be used to measure TH1/TH2/TH17/TH21 responses generated by CIN-3; Virus Batch #01-16C3 infection.

Stool Collection for cytokine levels

Cytokine levels will be determined by PCR.

Innate immune responses of PBMC-derived DCs against NoVs

Peripheral blood mononuclear cells (PBMCs) isolated from subjects before challenge with NoVs will be studied for induction of dendritic cells (DCs) following stimulation with human granulocyte/macrophage colony stimulating factor (GM-CSF) and Interleukin-4 (IL-4). DCs will be analyzed phenotypically by flow cytometry.

Adaptive Immune Responses in NoV Infected Individuals

Functional analysis of P-particle specific T cells (production of cytokines such as interleukin-2, interferon-γ and tumor necrosis factor-α, etc.) will be performed by stimulation with synthesized $CD4^+$ or $CD8^+$ T cell epitopes *in vitro*.

Characterization of Gut Microbiome

Total Deoxyribonucleic Acid (DNA) will be isolated from stool specimens using the NucliSENS EasyMag automated nucleic acid extraction system (BioMerieux). 16s rRNA sequences will be amplified by polymerase chain reaction using a universal primer set that detects all bacterial species. The resulting amplicons will be sequenced using next generation sequencing systems.

7.2.1 Laboratory Specimen Preparation, Handling, and Storage

Whole blood will be collected by the venous route using aseptic techniques. Depending on the testing performed, aliquots of blood will be sent to the CCHMC Clinical Laboratory and/or the Laboratory for Specialized Clinical Studies, Division of Infectious Diseases, Cincinnati Children's Hospital Research Foundation for processing.

Stool samples will be collected using specially designed stool collection kits. Study team members will use appropriate contact isolation procedures to minimize their risk of handling the stool specimens. If visible spillage is present on the outside of the stool container, the container will be wiped with an appropriate disinfectant and dried prior to the study labels being placed on the tubes. Any spillage on the tubes will be handled in the same manner. The samples will then be placed in sealed specimen bags and sent to the Laboratory for Specialized Clinical Studies, Division of Infectious Diseases, Cincinnati Children's Hospital Research Foundation, for processing and storage until testing is performed. Instructions for Specimen Preparation, Handling, and Storage Instructions for specimen preparation, handling, and storage are included in the protocol-specific MOP. Instructions for specimen shipment are included in the protocol.

7.2.2 Laboratory Specimen Shipping

Instructions for Specimen Preparation, Handling, and Storage Instructions for specimen preparation, handling, and storage are included in the protocol-specific MOP. Instructions for specimen shipment are included in the protocol-specific MOP.

8 ASSESSMENT OF SAFETY

8.1 Assessing and Recording Safety Parameters

8.1.1 Adverse Events (AEs)

ICH E6 defines an AE as any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product regardless of its causal relationship to the study treatment. FDA defines an AE as any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related.

An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of medicinal (investigational) product. The occurrence of an AE may come to the attention of study personnel during study visits and interviews of a study recipient presenting for medical care, or upon review by a study monitor.

AEs, including solicited systemic (subjective and quantitative) reactions, will be captured on the appropriate data collection form and eCRF. Information to be collected for unsolicited non-serious AEs includes event description, date of onset, assessment of severity, relationship to study product and alternate etiology (assessed only by those with the training and authority to make a diagnosis and listed on the Form FDA 1572 as an investigator), date of resolution, seriousness and outcome. AEs occurring during the trial collection and reporting period will be documented appropriately regardless of relationship. AEs will be followed through resolution.

Any medical condition that is present at the time that the subject is screened will be considered as baseline and not reported as an AE. However, if the severity of any pre-existing medical condition increases, it should be recorded as an AE.

If an event meets both the criteria of a study endpoint and an adverse event, the event only will be reported as a study endpoint.

All AEs (laboratory and clinical symptoms) will be graded for severity and assessed for relationship to norovirus (see definitions). AEs characterized as intermittent require documentation of onset and duration of each episode. The start and stop date of each reported AE will be recorded on the appropriate data collection form and eCRF. Changes in the severity of an AE will be documented to allow an assessment of the duration of the event at each level of intensity.

Severity of Event:

All AEs (laboratory and clinical symptoms) will be assessed by the investigator 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 (Grade 1): Events that are usually transient and may require only minimal or no treatment or therapeutic intervention and generally do not interfere with the subject's usual activities of daily living.
- Moderate (Grade 2): Events that are usually alleviated with additional specific therapeutic intervention. The event interferes with usual activities of daily living, causing discomfort but poses no significant or permanent risk of harm to the research subject.
- Severe (Grade 3): Events interrupt usual activities of daily living, or significantly affects clinical status, or may require intensive therapeutic intervention. Severe events are usually incapacitating.

Relationship to Study Products:

The assessment of the relationship of an AE to the administration of study product is made only by those with the training and authority to make a diagnosis and listed on the Form FDA 1572 as an investigator based on all available information at the time of the completion of the eCRF. Whether the AE is related or not, is not a factor in determining what is or is not reported in this trial. If there is any doubt as to whether a clinical observation is an AE, the event should be reported.

In a clinical trial, the study product must always be suspect. To help assess, the following guidelines are used.

- Related – There is a reasonable possibility that the study product caused the adverse event. Reasonable possibility means that there is evidence to suggest a causal relationship between the study product and the adverse event.
- Not Related – There is not a reasonable possibility that the administration of the study product caused the event.

8.1.2 Solicited Adverse Events

Solicited Adverse Events are adverse events that are common and known to or expected to occur following the administration of the study product. Subjects will be assessed for solicited adverse events following challenge administration and will complete an illness/solicited adverse events memory aid to record symptoms for five days after discharge from the isolation facility. Subjects will also be asked to report any unsolicited adverse events through Day 30 after study product administration. The subject will record the presence and severity, headache, nausea, vomiting, abdominal cramps/discomfort/pain, abdominal gurgling, abdominal bloating, myalgia, malaise/fatigue, anorexia/loss of appetite, and chills. The subjects will be provided with a thermometer. Any symptoms still present after Study Day 10 will continue to be followed until resolution or determined to be stable per investigator discretion.

Subjects will also be asked to report any medications taken for approximately 30 days following NoV challenge administration and any AEs including any emergency room or other medical visits (other than routine check-ups). The subject illness/solicited adverse events and unsolicited adverse event(s) memory aids will be reviewed with the subject at subsequent visits. Illness and solicited adverse events will be analyzed using Appendix E.

Severity of solicited adverse events will be graded according to Appendix E: Solicited Adverse Events Grading Scale.

8.1.3 Serious Adverse Events (SAEs)

An adverse event or suspected adverse reaction is considered “serious” if, in the view of either the investigator or sponsor, it results in any of the following outcomes:

- death
- a life-threatening adverse event
- inpatient hospitalization or prolongation of existing hospitalization (excluding any extended stay during challenge admission due to continued vomiting, diarrhea, etc.)
- a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions, or
- a congenital anomaly/birth defect

Important medical events that may not result in death, be life-threatening, or require hospitalizations may be considered serious when, based upon appropriate medical judgment they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. 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.

Life-threatening adverse event. An adverse event is considered "life-threatening" if, in the view of either the site principal investigator or sponsor, its occurrence places the patient or subject at immediate risk of death. It does not include an adverse event, had it occurred in a more severe form, might have caused death.

SAEs will be:

Assessed for severity and relationship to study product and alternate etiology (if not related to study product) by a licensed study physician listed on the Form FDA 1572 or by the Institution as the site Principal Investigator or Sub-Investigator.

Recorded on the appropriate SAE data collection form and eCRF.

Followed through resolution by a licensed study physician (for IND studies, a physician listed on the Form FDA 1572 as the site Principal Investigator or Sub-Investigator).

Reviewed and evaluated by an Independent Safety Monitor (ISM) (as deemed necessary), the SMC (periodic review unless related), DMID, and the IRB.

8.1.4 New-Onset Chronic Medical Conditions (NOCMCs)

NOCMCs are defined as any new ICD-10 diagnosis that is applied to the subject during the duration of the study, after receipt of the study agent, that is expected to continue for at least 3 months and requires continued health care intervention.

NOCMCs will be:

Assessed for severity and relationship to study product and alternate etiology (if not related to study product) by a licensed study physician listed on the Form FDA 1572 or by the Institution as the site Principal Investigator or Sub-Investigator.

Recorded on the appropriate data collection form and eCRF.

Followed through resolution by a licensed study physician (for IND studies, a physician listed on the Form FDA 1572 as the site Principal Investigator or Sub-Investigator).

Reviewed and evaluated by an Independent Safety Monitor (ISM) (as deemed necessary), the SMC (periodic review unless related), DMID, and the IRB.

8.2 Specification of Safety Parameters

Safety will be based on the frequency and severity of non-norovirus challenge-related serious adverse events occurring throughout the course of the study.

Solicited Adverse Events – solicited/anticipated events following challenge administration.

Unsolicited Adverse Events- non-Norovirus challenge-related non-serious events occurring during 30 days following challenge administration.

Clinical safety laboratory adverse events to include WBC, absolute neutrophil count, hemoglobin, platelet count, potassium, sodium, creatinine, ALT and total bilirubin.

Safety will be assessed by frequency and incidence of solicited and unsolicited AE/SAEs (laboratory and clinical symptoms) in each group. A Safety Monitoring Committee (SMC) will be convened to review subject safety data which may include solicited and unsolicited AE/SAEs, concomitant medications, clinical laboratory values and any physical examinations. Safety data will be reviewed by the SMC per the SMC charter for this study.

The study Sponsor may interrupt study dosing and/or study entry at any time if medically indicated. To minimize risk, the study enrollment and dosing will be stopped, and the safety data will be reviewed by the SMC, if any of the following Halting Rules has been met.

8.3 Reporting Procedures

Adverse Events will be collected from Day 1 through Day 30 post challenge.

Serious Adverse Events, including deaths and life-threatening events, will be collected from Day 1 through Day 180 post challenge.

New-Onset Chronic Medical Conditions (NOCMCs) will be collected from Day 1 following receipt of the study agent through Day 180 post challenge.

Any adverse event that meets a protocol-defined serious criterion must be submitted immediately (within 24 hours of site awareness) on an SAE form to the CCHMC IRB as well as other required reporting agencies.

8.3.1 Reporting Serious Adverse Events

SAEs will be followed until resolution even if this extends beyond the study-reporting period. Resolution of an AE is defined as the return to pretreatment status or stabilization of the condition with the expectation that it will remain chronic.

Any AE that meets a protocol-defined serious criterion must be submitted immediately (within 24 hours of site awareness) on an SAE form to the DMID Pharmacovigilance Group, at the following address:

DMID Pharmacovigilance Group

Clinical Research Operations and Management Support (CROMS)

6500 Rock Spring Dr. Suite 650

Bethesda, MD 20817, USA

SAE Hot Line: 1-800-537-9979 (US) or 1-301-897-1709 (outside US)

SAE FAX Number: 1-800-275-7619 (US) or 1-301-897-1710 (outside US)

SAE Email Address: PVG@dmidcroms.com

In addition to the SAE form, select SAE data fields must also be entered into the DCC system (for example: Advantage eClinical®). Please see the protocol-specific MOP for details regarding this procedure.

Other supporting documentation of the event may be requested by the DMID Pharmacovigilance Group and should be provided as soon as possible.

The site will send a copy of the SAE report(s) to the ISM (as deemed necessary) when they are provided to the DMID Pharmacovigilance Group. The DMID Medical Monitor and DMID Clinical Project Manager will be notified of the SAE by the DMID Pharmacovigilance Group. The DMID Medical Monitor will review and assess the SAE for regulatory reporting and potential impact on study subject safety and protocol conduct.

At any time after completion of the study, if the site principal investigator or appropriate sub-investigator becomes aware of an SAE that is suspected to be related to study product, the site principal investigator or appropriate sub-investigator will report the event to the DMID Pharmacovigilance Group.

8.3.2 Regulatory Reporting for Studies Conducted Under DMID-Sponsored IND

Following notification from the investigator, DMID, the Investigational New Drug (IND) sponsor, will report any suspected adverse reaction that is both serious and unexpected. DMID will report an adverse event as a suspected adverse reaction only if there is evidence to suggest a causal relationship between the drug and the adverse event. DMID will notify FDA and all participating investigators (i.e., all investigators to whom the sponsor is providing drug under its IND(s) or under any principal investigator's IND(s)) in an IND safety report of potential serious risks from clinical trials or any other source, as soon as possible, but in no case later than 15 calendar days after the sponsor determines that the information qualifies for reporting as specified in 21 CFR Part 312.32. DMID will also notify FDA of any unexpected fatal or life-threatening suspected adverse reaction as soon as possible, but in no case later than 7 calendar days after the sponsor's initial receipt of the information. Relevant follow up information to an IND safety report will be submitted as soon as the information is available. Upon request from FDA, DMID will submit to FDA any additional data or information that the agency deems necessary, as soon as possible, but in no case later than 15 calendar days after receiving the request.

All serious events designated as "not related" to study product(s), will be reported to the FDA at least annually in a summary format.

8.3.3 Regulatory Reporting for Studies Not Conducted Under DMID-Sponsored IND

Following notification from the investigator, the IND sponsor will report events that are both serious and unexpected that are related to study product(s) to the FDA within the required timelines as specified in 21 CFR Part 312.32: fatal and life-threatening events within 7 calendar days (by telephone or fax). All written reports will be sent to the FDA within 15 calendar days. All serious events designated as "not related" to study product(s), will be reported to the FDA at least annually in a summary format.

8.3.4 Reporting of Pregnancy

Pregnancies occurring in study subjects will be reported in IDES. With the subject's permission all study mandated venous blood samples will be collected and the subject will continue in

follow-up for safety events. Efforts will be made to follow all pregnancies reported during the course of the study to pregnancy outcome with the subject's permission.

8.4 Type and Duration of Follow-up of Subjects after Adverse Events

All adverse and serious adverse events will be followed to resolution or until determined to be stable.

AEs will be assessed and followed from initial recognition of the AE through end of the protocol defined follow-up period.

SAEs will be followed up through resolution even if duration of follow-up goes beyond the protocol-defined follow-up period.

Resolution of an AE is defined as the return to pre-treatment status or stabilization of the condition with the expectation that it will remain chronic.

8.5 Procedures to be Followed in the Event of Abnormal Laboratory Test Values or Abnormal Clinical Findings

The site principal investigator or appropriate sub-investigator is responsible for recording all AE/SAEs that are observed or reported during this trial, regardless of the relationship to study product. AE/SAEs, abnormal laboratory test values, or abnormal clinical findings will be collected, assessed, documented, reported, and followed appropriately.

For baseline laboratory results that are abnormal according to the local laboratory reference range and fall within Grade 1 toxicity table range, these will not be considered laboratory adverse event (AE) and will thus not be graded. However, if baseline clinical labs fall within Grade 1 range, then a laboratory AE is reported only if the value changes such that it falls into Grade 2 or higher when subsequent safety laboratory testing is done.

8.6 Halting Rules

8.6.1 Study Halting Criteria

DMID, the study Sponsor, may interrupt study dosing and/or study entry at any time if medically indicated. No additional subjects will be dosed until safety review has been completed. To minimize risk, the study enrollment and dosing will be stopped and the safety data will be reviewed by the SMC, if any of the following Halting Rules have been met at any time throughout the study:

1. Death of an enrolled subject or any SAE if the SAE is considered by DMID Medical Monitor to be related to the study product.
2. Any Grade 3 or higher adverse event (of the same type, per MedDRA Preferred Term or parameter, excluding diarrhea, vomiting, nausea, abdominal cramping/discomfort, and other solicited adverse events listed in section 8.1.2 as these are expected) in two subjects.

The objective of the SMC is to decide if the study should continue per protocol, proceed with caution, be further investigated, be discontinued, or be modified and then proceed. Suspension of enrollment (for a particular group or for the entire study) is another potential outcome of a safety review.

Upon completion of this review and receipt of the SMC recommendation, DMID will determine if study entry or study dosing should be interrupted or if study entry and study dosing may continue according to the protocol.

The study may also be suspended because of safety findings such as an overall pattern of symptomatic, clinical, or laboratory events that the Medical Monitor considers related to study product and that may appear minor in terms of individual events, but that may collectively represent a serious potential concern for safety.

8.7 Safety Oversight (ISM plus SMC)

8.7.1 Independent Safety Monitor (ISM)

An Independent Safety Monitor will be appointed to this study. Independent Safety Monitors are physicians with relevant expertise and whose primary responsibility is to provide timely independent safety monitoring. An ISM is assigned to each study site, is in close proximity to the study site and has the authority to readily access study subject records. The ISM reviews any SAE that occurs at the study site in real time and provides an assessment to the IND sponsor.

8.7.2 Safety Monitoring Committee (SMC)

This clinical study will utilize an SMC, which is an independent group of experts that advises the DMID. The primary responsibility of the SMC is to monitor subject safety. The SMC is external to the DMID and comprises at least 3 voting members. The SMC will consist of members with appropriate phase 1 study expertise to contribute to the interpretation of the data from this trial. Its activities will be delineated in a SMC charter that will describe the membership, responsibilities, and the scope and frequency of data reviews. The SMC will operate on a

conflict-free basis independently of the study team. The DMID or the SMC may convene ad hoc meetings of the SMC according to protocol criteria or if there are concerns that arise during the study.

As defined in the charter, the SMC will review data at specified times during the course of the study for subject and overall study progress and will conduct ad hoc reviews as appropriate when a halting rule is met or for immediate concerns regarding observations during this study. It is anticipated the SMC will have planned meetings at the following time points:

- Before study initiation
- After each challenge cohort has been completed
- Ad hoc when a halting rule is met or as needed
- At least annually

If an Ad hoc meeting were convened, the SMC may be asked to review solicited and unsolicited AE/SAEs, concomitant medications, clinical laboratory values and any physical examinations. Safety data will be reviewed by the SMC per the SMC charter for this study.

Final review meeting: 6 to 8 months after clinical database lock to review the cumulative unblinded safety data for the study. The data will be provided in a standard summary format. The SMC may be asked to provide recommendations in response to questions posed by the IND sponsor.

Procedures for SMC reviews/meetings will be defined in a charter. The SMC will review applicable data to include but not limited to enrollment, demographic, dosing, laboratory and safety data which may include solicited and unsolicited AE/SAEs, concomitant medications, clinical laboratory values and any physical examinations. Safety data will be reviewed by the SMC per the SMC charter for this study. As an outcome of each review/meeting, the SMC will make a recommendation as to the advisability to continue, modify or terminate the study.

The IND sponsor or the SMC chair may convene the SMC on an ad hoc basis according to protocol criteria or if there are immediate concerns regarding observations during the course of the study. The Medical Monitor is empowered to stop study enrollment and/or study procedures if adverse events that meet the halting criteria are reported. The Medical monitor and the ISM will be responsible for reviewing SAEs in real time. The SMC will review SAEs on a regular basis and ad hoc during the study.

9 HUMAN SUBJECTS PROTECTION

9.1 Institutional Review Board/Independent Ethics Committee

Each site principal investigator will obtain IRB approval for this protocol to be conducted at his/her research site(s) and send supporting documentation to the DMID before initiating recruitment of subjects. The investigator will submit applicable information to the IRB/IEC on which it relies for the review, to conduct the review in accordance with 45 CFR 46, ICH E6 GCP, and as applicable, 21 CFR 56 (Institutional Review Boards) and 21 CFR 50 (Protection of Human Subjects), other federal, state, and local regulations. The IRB/IEC must be registered with OHRP as applicable to the research. DMID must receive the documentation that verifies IRB/IEC-approval for this protocol, associated informed consent documents, and upon request any recruitment material and handouts or surveys intended for the subjects, prior to the recruitment and enrollment of subjects.

Any amendments to the protocol or consent materials will be approved by the IRB/IEC before they are implemented. IRB/IEC review and approval will occur at least annually throughout the enrollment and follow-up of subjects and may cease if annual review is no longer required by applicable regulations and the IRB/IEC. The investigator will notify the IRB/IEC of deviations from the protocol and reportable SAEs, as applicable to the IRB/IEC policy.

Each institution engaged in this research will hold a current Federalwide Assurance (FWA) issued by the Office of Human Research Protection (OHRP) for federally funded research.

9.2 Informed Consent Process

Informed consent is a process that is initiated prior to an individual agreeing to participate in a trial and continuing throughout the individual's trial participation. Before any study procedures are performed, informed consent will be obtained and documented. Subjects will receive a concise and focused presentation of key information about the clinical trial, verbally and with a consent form. The explanation will be organized and presented in lay terminology and language that facilitates understanding why one might or might not want to participate.

An investigator or designee will describe the protocol to potential subjects face-to-face. The key information about the purpose of the study, the procedures and experimental aspects of the study, risks and discomforts, any expected benefits to the subject, and alternative treatment will be presented first to the subject.

Subjects will also receive an explanation that the trial involves research, and a detailed summary of the proposed study procedures and study interventions/products. This will include aspects of the trial that are experimental, the probability for random assignment to treatment groups, any expected benefits, all possible risks (including a statement that the particular treatment or procedure may involve risks to the subject or to the embryo or fetus, if the subject is or may become pregnant, that are currently unforeseeable), the expected duration of the subject's participation in the trial, alternative procedures that may be available and the important potential benefits and risks of these available alternative procedures.

Subjects will be informed that they will be notified in a timely manner if information becomes available that may be relevant to their willingness to continue participation in the trial. Subjects will receive an explanation as to whether any compensation and any medical treatments are available if injury occurs, and, if so, what they consist of, or where further information may be obtained. Subjects will be informed of the anticipated financial expenses, if any, to the subject for participating in the trial, as well as any anticipated prorated payments, if any, to the subject for participating in the trial. They will be informed of whom to contact (e.g., the investigator) for answers to any questions relating to the research project.

Information will also include the foreseeable circumstances and/or reasons under which the subject's participation in the trial may be terminated. The subjects will be informed that participation is voluntary and that they are free to withdraw from the study for any reason at any time without penalty or loss of benefits to which the subject is otherwise entitled.

The extent of the confidentiality of the subjects' records will be defined, and subjects will be informed that applicable data protection legislation will be followed. The sponsoring company and the participant's personal health care provider may review parts of participant records. Subjects will be informed that the monitor(s), auditors(s), IRB, NIAID, and regulatory authority(ies) will be granted direct access to the subject's original medical records for verification of clinical trial procedures and/or data without violating the confidentiality of the subject, to the extent permitted by the applicable laws and regulations, and that, by signing an informed consent form, the subject is authorizing such access.

Subjects will be informed that records identifying the subject will be kept confidential, and, to the extent permitted by the applicable laws and/or regulations, will not be made publicly available and, if the results of the trial are published, the subject's identity will remain confidential. Subjects will be informed whether private information collected from this research and/or specimens will be used for additional research, even if identifiers are removed.

Subjects will be allowed sufficient time to consider participation in this research trial and have the opportunity to discuss this trial with their family, friends or legally authorized representative, or think about it prior to agreeing to participate.

Informed consent forms will be IRB-approved, and subjects will be asked to read and review the consent form. Subjects must sign the informed consent form prior to starting any study procedures being done specifically for this trial.

Once signed, a copy of the informed consent form will be given to the subject(s) for their records. The subject(s) may withdraw consent at any time throughout the course of the trial. The rights and welfare of the subject(s) 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.

Study personnel may employ recruitment efforts prior to obtaining study consent if a patient-specific screening consent is on record or if the IRB has agreed that chart review is allowed without a fully executed screening consent. In cases where there is not a patient-specific screening consent on record, site clinical staff may pre-screen via chart review and refer potential subjects to the Research staff. Research staff would obtain consent per the standard informed consent process before conducting protocol-specific screening activities.

New information will be communicated by the site principal investigator to subjects who consent to participate in this trial in accordance with IRB requirements. The informed consent document will be updated, and subjects will be re-consented per IRB requirements, if necessary. Subjects will be given a copy of all informed consent forms that they sign.

9.3 Consent for Future Use of Stored Specimens and Data

Subjects who enroll will have any remaining stool and remaining serum from venous blood samples for possible use in future research studies, such as examining additional immunological assessments or testing for antibodies against other viruses or bacteria. These residual clinical samples will be stored indefinitely at a central clinical storage facility and may be shared with investigators at the participating site and with other investigators at other institutions.

Residual clinical samples will be available upon the completion of the study; however, future use clinical samples may be requested from DMID and shipped from the DMID CAR at any time.

The samples will not be sold or used directly for production of any commercial product. Each sample will be encoded (labeled) only with a barcode and a unique tracking number to protect subject confidentiality.

There are no benefits to subjects in the collection, storage and subsequent use of their specimens for future research. Reports about future research done with subjects' samples will NOT be kept in their health records.

Subjects enrolled into the study will have their samples saved to be used for future research.

Each sample will be encoded (labeled) only with a barcode and a unique tracking number to protect subject's confidentiality. There are no benefits to subjects in the collection, storage and subsequent research use of specimens. Reports about future research done with subject's samples will not be kept in their health records.

Future Use of Stored Specimens

Residual samples are those that are left over after the study has been completed and are stored for possible use in future research studies. Future use samples are extra tube/s of blood collected and stored for possible use in future research studies. Retention of residual and future use samples and the potential for use in future research studies will be a condition of study participation. Subjects who sign the informed consent form for the study are consenting to allow the collection, storage and use of any residual samples (serum or cells derived from venous blood samples) or future use samples for possible use in future research studies, such as examining additional immunological assessments or testing for antibodies against other viruses or bacteria.

Residual and future use samples will be encoded with a barcode label and unique tracking number to protect subject identity. Samples will be stored indefinitely at a DMID designated central storage facility. Residual samples may be shared for purposes other than per protocol analysis with investigators at participating VTEU sites and with investigators at other institutions once the clinical study report has been finalized. Future use research samples may be requested from DMID and shipped from the DMID CMS at any time.

Residual and future use samples will not be sold or used directly for production of any commercial product. Genetic tests may be performed on samples. There are no benefits to subjects in the collection, storage and subsequent use of their specimens for future research. Reports about future research done with subjects' samples will NOT be kept in their health records.

Subjects may withdraw consent for study participation at any time by notifying the study investigators or study staff in writing. However, any data from sample(s) collected prior to the withdrawn consent will not be removed including genomic data. There will be no further use of residual samples or collection and use of future use samples after consent has been withdrawn.

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

This trial will be inclusive of all adults who meet the Subject Inclusion Criteria (see Section 5.1.1) and do not meet the Subject Exclusion Criteria (see Section 5.1.2), regardless of religion, sex, or ethnic background. Non-English speakers will be excluded from this protocol as it is medically necessary that the subjects are able to communicate fluently with the medical staff regarding acute medical changes. Should the outcome of this trial be deemed acceptable, additional trials may be initiated including those in other populations.

Pregnant women will be excluded as it is unknown if the challenge poses risks to an unborn child. Children are excluded due to the ethical considerations of not enrolling a child in a greater than minimal risk study unless there is a prospect for direct benefit.

9.5 Subject Confidentiality

Subject confidentiality is strictly held in trust by the participating investigators, their staff, and the sponsor(s) and their agents. This confidentiality includes documentation, investigation data, subject's clinical information, and all other information generated during participation in the study. No information concerning the study, or the data generated from the study will be released to any unauthorized third party without prior written approval of the DMID and the subject. Subject confidentiality will be maintained when study results are published or discussed in conferences. The sponsoring company and the participant's personal health care provider may review parts of participant records. The study monitor, or other authorized representatives of the sponsor or governmental regulatory agencies 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.

All records will be kept locked and all computer entry and networking programs will be carried out with coded numbers only and with password protected systems. All non-clinical specimens, evaluation forms, reports, and other records that leave the site will be identified only by a coded number.

Each subject will be assigned a unique study identifier. All data collection sheets will identify the subject by a unique identifier, and the date. Names will not be used on any samples or in any publication of this study. All efforts will be made to protect the privacy of subjects.

This information and data will not be used by the site principal investigator or other study personnel for any purpose other than conducting the study. These restrictions do not apply to: (1) information which becomes publicly available through no fault of the site principal investigator or other study personnel; (2) information which is necessary to disclose in confidence to an IRB solely for the evaluation of the study; (3) information which it is necessary to disclose in order to provide appropriate medical care to a study subject; or (4) study results which may be published.

9.6 Costs, Subject Compensation, and Research Related Injuries

There is no cost to subjects for the research tests, procedures, and study product while taking part in this trial. Procedures and treatment for clinical care may be billed to the subject, subject's insurance or third party. Subjects may be compensated for their participation in this trial.

Compensation will be in accordance with the local IRB's policies and procedures, and subject to IRB approval.

If it is determined by the site principal investigator that an injury occurred to a subject as a direct result of the tests or treatments that are done for this trial, then referrals to appropriate health care facilities will be provided to the subject. Study personnel will try to reduce, control, and treat any complications from this trial. Immediate medical treatment may be provided by the participating site. No financial compensation will be provided to the subject by the NIAID, NIH to the subject, for any injury suffered due to participation in this trial.

There is no cost to subjects for the research tests, procedures, and study product while taking part in this trial. Subjects may be compensated for their participation in this trial. Compensation will be in accordance with the local IRB's policies and procedures, and subject to IRB approval.

If it is determined by the site principal investigator that an injury occurred to a subject as a direct result of the tests or treatments that are done for this trial, then referrals to appropriate health care facilities will be provided to the subject. Study personnel will try to reduce, control, and treat any complications from this trial. Immediate medical treatment may be provided by the participating site. No financial compensation will be provided to the subject by the NIAID, NIH to the subject, or by the participating site for any injury suffered due to participation in this trial.

10 STATISTICAL CONSIDERATIONS

10.1 Study Hypotheses

This is a phase 1 dose-escalation study designed to determine the optimal dose of an experimental human norovirus GII.4 challenge strain to be used in future trials testing candidate vaccines and/or therapeutics. The trial is exploratory in nature and thus, is not designed to test a specific hypothesis. Rather, it is intended to estimate illness (and therefore, infection) rates for up to three different challenge doses and to evaluate the safety of the challenge strain.

10.2 Sample Size Considerations

The study will enroll up to 3 cohorts of approximately 16 subjects each, for a total of up to 48 subjects, aged 18-49 years (inclusive). Each cohort will enroll 15 secretor positive subjects and 1 secretor negative subjects. Based on previous studies conducted using the GII.4 norovirus study product, it is not expected that secretor negative subjects will become infected, and therefore they serve as controls for the secretor positive subjects.

The sample size of approximately 16 per dose group was chosen for feasibility based on the size of the inpatient unit that will be utilized for this study.

Based on results of earlier studies the first cohort will receive a dose of $3.5x10^3$ copies. Subsequent doses will be based on attainment of response rate $\geq$50%. They will be smaller if the threshold of 50% is observed and will be larger if it is not observed. If the illness rate is $\geq$50% for this cohort then the final cohort will be tested at $3.5x10^1$ copies, the lower bound of the range of consideration.

The final row of the schema (Figure 1) indicates possible doses for future studies based on the results of this study. It will not be sufficient to demonstrate a 50% response rate (8/16) at the final dose as this yields a confidence interval of (26% to 74%). A response rate of 72% (14/18) is required for the lower bound of the 95% confidence interval to exclude 50% (14/18 implies a confidence interval of 52.3% to 93.6%).

Table 2 below shows the precision of the estimated infection rates for a range of possible infection rates that could be observed in the secretor positive subjects in one cohort.

Table 3. Observed illness rates and 95% confidence intervals for a challenge dose group of N=18 secretor positive subjects.

Number with illness	Observed Illness Rate	95% Exact CI
0	0	(0.00, 0.19)
2	0.11	(0.01, 0.35)
4	0.22	(0.06, 0.48)
6	0.33	(0.13, 0.59)
8	0.44	(0.22, 0.69)
10	0.56	(0.31, 0.78)
12	0.67	(0.41, 0.87)
14	0.78	(0.52, 0.94)
16	0.89	(0.65, 0.99)
18	1.00	(0.81, 1.00)

10.3 Treatment Assignment Procedures

10.3.1 Randomization Procedures

This is a non-randomized dose escalation study. Enrollment will be done online using the enrollment module of the Data Coordinating Center's (DCC) Internet Data Entry System (IDES).

Each cohort will be comprised of approximately 16 subjects (15 secretors and 1 non-secretors) who will receive the same dose of challenge product. Each cohort will have 2-3 additional subjects to serve as alternates. At least 1 alternate will be a secretor and 1 a non-secretor and if needed, they will fill the role of the subject who dropped from the primary subject list.

Instructions for use of the enrollment module will be included in the IDES User's Guide. Manual back-up procedures and instructions are provided for use in the event that the site temporarily loses access to the Internet or the online enrollment system is unavailable.

Per International Conference on Harmonisation (ICH) guideline E6: Good Clinical Practice (GCP), screening records will be kept at each participating clinical study site to document the reason why an individual was screened but failed trial entry criteria. The reasons why individuals failed screening will be recorded in IDES.

10.3.2 Masking Procedures

An unblinded study member will review the "secretor" status of subjects and prepare a list of subjects to be admitted to the inpatient unit for challenge. The subjects, the study personnel who perform any study-related assessments, and laboratory personnel performing study assays will be blinded to secretor status.

10.4 Planned Interim Analyses

10.4.1 Interim Safety Review

An SMC will be convened by DMID to review subject safety data, which may include solicited and unsolicited AE/SAEs, concomitant medications, clinical laboratory values, and any physical examinations. Safety data will be reviewed by the SMC as detailed in Section 8.7 and per the SMC charter for this study. As an outcome of each review/meeting, the SMC will make a recommendation as to the advisability of proceeding with the next cohort, and to continue, modify, or terminate the study.

10.4.2 Interim Immunogenicity or Efficacy Review

As part of the SMC review between cohorts, the number of subjects who achieve illness will be presented in order to determine which dose to administer in the following cohort.

An interim immunogenicity review will be performed after the second cohort to determine if there is a correlation between anti- norovirus GII.4 serum IgG by ELISA and/or blocking assay (protective antibody titers) and illness rate. If anti-norovirus GII.4 serum IgG by ELISA and/or blocking assay correlates to incidence of illness, then the eligibility criteria will be revised for the final cohort to exclude people at low risk for norovirus infection.

Summaries for these reviews will be included in the unblinded report prepared for the closed session of the SMC. No formal hypothesis testing will be performed.

10.5 Final Analysis Plan

A formal SAP will be developed and finalized prior to database lock.

The final analysis will be performed, and clinical study report completed and distributed when all primary and secondary endpoint data are available. Any available data from exploratory endpoints may also be included. The clinical study report will be amended as the additional exploratory endpoint data become available. For purposes of scientific meeting presentation of

study progress in advance of the final analysis, the protocol PI may request analysis of the demographics and blinded aggregate analyses of available primary and secondary endpoint data.

10.5.1 Analysis Populations

The Safety Analysis population includes all subjects who received at least a partial dose of the challenge study product.

The modified intent-to-treat (mITT) population includes all subjects who received the challenge study product and contributed both pre- and at least one post-challenge sample during the inpatient period for which a conclusion can be made regarding illness and infectivity.

The per protocol (PP) population includes all subjects in the mITT subset with the following exclusions:

- Data from all available visits for subjects found to be ineligible at baseline
- Data from all available visits for subjects who do not ingest all of the inoculum
- Data from all visits subsequent to protocol deviations, such as:
    - Receipt of non-study licensed live vaccine through Day 30
    - Long-term use (≥2 weeks) of high-dose oral (≥20 mg per day prednisone or equivalent) or parenteral glucocorticoids, or high-dose inhaled steroids for greater than 7 days
    - Receipt of experimental products through Day 180
    - Receipt of parenteral immunoglobulin or blood products within 3 months post-challenge
- Data from any visit that occurs substantially out of window

10.5.2 Primary Objectives

Determine the optimal challenge dose of Norovirus GII.4 CIN-3 Batch No.: 01-16C3 norovirus to achieve illness in ≥50% of subjects (illness is defined as norovirus infection determined by positive PCR and either a) ≥3 loose or liquid stools in a 24-hour period, b) ≥300 gm of loose or liquid stool in a 24-hour period c) and/or any episode of vomiting), during the inpatient period.

The primary objective of this study is to find the lowest dose of the GII.4 challenge strain capable of producing at least a 50% illness rate in secretor positive subjects. The undiluted stock of the CIN-3 challenge strain is $3.5x10^5$ copies/mL. Based on previous experience with this GII.4

norovirus, the dose of norovirus to be administered to the first cohort is $3.5x10^3$ copies. This is a descriptive study and is not intended to test any formal hypothesis.

The schematic in Figure 1 is intended to guide the selection of doses to be tested and the final dose to be adopted for future trials. It is based on the observation that in previous studies with CIN-1 the illness response rates were relatively "flat". That is, a reduction in dose did not result in an equivalent reduction in response. Results in this trial may be inconsistent with this assumption and the proposed schema may be modified based on observed results. Unless explicitly stated, response rates will refer only to secretor positive subjects receiving an active challenge dose.

A logistic regression model of responses as a function of log dose will be constructed to select a recommended dose for future studies.

The challenge results will also be described by cohort and dose group (combining like doses) using descriptive statistics. In particular, point estimates and 95% confidence intervals will be computed.

10.5.3 Secondary Objectives

Evaluate the safety of the Norovirus GII.4 CIN-3 Batch No.:01-16C3 challenge strain

Solicited events will be collected through Study Day 10 after virus challenge. These results will be summarized by taking subjects' maximum response for each solicited event category and for all categories combined. These results will be tabulated by dose and dichotomized into none versus mild, moderate or severe and examined for a dose-response relationship using logistic regression.

Unsolicited events, in contrast to solicited events, will be based on subjects experiencing Grade 3 adverse events after virus challenge that are not norovirus related. The rate of such AEs is expected to be low. If this is the case then this data will be summarized using a listing of all events, including, but not necessarily limited to, a description of the event, the study cohort, challenge dose and timing of the event. If there are a sufficient number of events they will be summarized by challenge dose and formal comparisons will be made.

Determine the rate of infection at different challenge doses by:

- Detection of Norovirus GII.4 in the stool using specific qRT-PCR
- Anti- Norovirus GII.4 serum IgG by ELISA (≥4-fold rise from baseline through Day 30)

The number and percentage of subjects with infection will be described by cohort and dose group (combining like doses) using descriptive statistics. In particular, point estimates and 95% confidence intervals will be computed.

A number of the secondary and exploratory objectives listed below will be summarized by examining the mean response. "Mean" should be understood to refer to the arithmetic mean or the geometric mean, as appropriate. The summary statistic will depend on the distribution of the particular parameter.

Measure the severity of acute gastroenteritis.

The modified Vesikari scores will be summarized by dose using descriptive statistics. The distribution of the scores will be examined, transformations such as a log transformation may be made, and appropriate point estimates and summary of variability will be provided. Scores will also be tested to examine if there is a relationship between Vesikari scores and challenge dose. The statistical method used (parametric or non-parametric) will depend on the distribution of the scores. The duration of vomiting and/or diarrhea (in hours) during the inpatient period will be calculated for each subject and summarized by dose using descriptive statistics.

Determine the quantity and duration of virus shedding in stool by qRT-PCR

PCR results will be summarized using estimates and 95% confidence limits for the percentage of subjects with positive stools, the mean duration (in days) of viral shedding, and the peak GEC/mL for each subject will be summarized. The results will be summarized for cohort and dose specific groups. Additionally, longitudinal methods may be used to explore the possibility of determining not just the presence of virus but to estimate trends in the magnitude of virus shed over time.

10.5.4 Exploratory Objectives

Determine norovirus GII.4 –specific antibody responses at baseline, Day 15, and Day 30

- Serum IgA and IgG by ELISA
- Serum Blocking Antibody by ELISA

Pre- and post-challenge virus-specific ELISA assay measures will be summarized using the geometric mean fold rise and proportion of subjects with at least a 4-fold antibody response for each of the assays.

Determine total and norovirus GII.4-specific Memory B cell response by ELISpot assay

The number of challenge virus-specific Memory B cells per 10^6 PBMC will be summarized by time point and cohort/dose groups.

Determine the effect of baseline norovirus antibody levels (serum IgG, IgA, and blocking antibody) on becoming infected with norovirus

This objective will be addressed by testing for a possible association between level of pre-existing antibodies, challenge dose and rates of illness or infection. These results will be examined graphically by looking at the distribution of pre-existing antibodies among infected (ill) and not infected (not ill) subjects within dose groups and across all dose groups. If there is any suggestion of a relationship between pre-existing antibody and response this will be modeled using logistic regression to estimate the relationship between infection (illness) rates and antibody level.

Determine total and norovirus GII.4 -specific IgA- and IgG-Antibody Secreting Cells by ELISpot assay and Antibody Lymphocyte Supernatant by ELISA

The number of challenge virus-specific IgA and IgG ASCs per total IgA and IgG ASCs will be assessed. The proportions of norovirus specific IgA- and IgG-ASCs may be compared between time points and to baseline levels for individual subjects, as well as between challenge dosing groups.

The ALS assay will measure the secretion of Norovirus-specific antibodies in cultures of PBMCs by ELISA and will be summarized using the geometric mean fold rise.

10.5.5 Handling Missing Data

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

11 ELECTRONIC CASE REPORT FORMS AND ACCESS TO SOURCE DATA/DOCUMENTS

The participating VTEU site will maintain appropriate medical and research records in compliance with ICH E6, Section 4.9 and regulatory and institutional requirements for the protection of confidentiality of subjects. Each site will permit authorized representatives of the DMID, its designees, and appropriate regulatory agencies to examine (and when required by applicable law, to copy) clinical records for the purposes of quality assurance reviews, audits, and evaluation of the study safety and progress. These representatives will be permitted access to all source data and source documents (paper and electronic), which include, but are not limited to, hospital records, clinical and office charts, laboratory notes, memoranda, 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. Source data are all information, original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial.

The study uses direct data entry for the participating clinic site and a web-based e-Memory aid for subjects. All eCRFs serve as the source documents. Subjects will be trained to use a database to complete a web based “e-Memory aid” and are expected to enter information in the e-Memory aid each day. Subjects using the e-Memory aid will also be provided a paper memory aid for their use in the event they are unable to access the web-based system. The subjects will be asked to enter the information from the paper memory aid into the e-Memory aid once they are able to access the web-based system. Web-based access can be provided to subjects while on site for study visits.

Subjects will record temperature, systemic symptoms, and any new medications used following challenge through Study Day 10. Subjects will report any unsolicited adverse event through the 30 days following challenge.

Subjects will be instructed to contact the clinic staff immediately if they experience significant symptoms at any time during the study, for prompt follow-up in real time. The study clinic will be alerted in real time of any potential solicited events of Grade 3 severity entered in the e-Memory Aid. An email alert will be sent to the clinic site and the Emmes study team. Within one business day of site awareness, the site must attempt to follow up with the subject on the severe

solicited event and send an email to Emmes confirming attempted follow up with the subject. Instructions for completing the e-Memory aid are provided in the MOP and in a separate e-Memory aid instructions document that will be provided to the subjects at each vaccination. The site staff must review the e-Memory aid information and interview the subject at the next scheduled visit. The subject-entered data will be available for review by the clinician during the clinical interview.

The site staff will be the data originators for the clinically reviewed data in Advantage eClinical® that will be used for the study endpoints. A list of all authorized site staff data originators will be included on the Study Personnel/Signature Responsibility List.

12 QUALITY CONTROL AND QUALITY ASSURANCE

Following a written DMID-accepted site quality management plan, each participating site and its subcontractors are responsible for conducting routine quality assurance (QA) and quality control (QC) activities to internally monitor study progress and protocol compliance. The site principal investigator will provide direct access to all study-related sites, source data/data collection forms, and reports for the purpose of monitoring and auditing by the sponsor, and inspection by local and regulatory authorities. The site principal investigator will ensure all study personnel are appropriately trained and applicable documentations are maintained on site.

The DCC will implement quality control procedures beginning with the data entry system and generate data quality control checks that will be run on the database. Any missing data or data anomalies will be communicated to the participating site(s) for clarification and resolution.

DMID-designated clinical monitors will verify that the clinical trial is conducted, and data are generated, documented (recorded), and reported in compliance with the protocol, ICH/GCP guidelines, and the applicable regulatory requirements. Clinical monitoring reports will be submitted to DMID.

13 DATA HANDLING AND RECORD KEEPING

13.1 Data Management Responsibilities

The investigator is responsible to ensure the accuracy, completeness, legibility, and timeliness of the data reported. All source documents should be completed in a neat, legible manner to ensure accurate interpretation of data. Black or blue permanent ink is required to ensure clarity of reproduced copies. When making changes or corrections, 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.

Electronic case report forms will be created by the SDCC to record and maintain data for each subject enrolled in this study.

The sponsor and/or its designee will provide guidance to the site principal investigators and other study personnel on making corrections to the data collection forms and eCRF.

13.2 Data Coordinating Center/Biostatistician Responsibilities

All electronic case report forms and laboratory reports must be reviewed by the clinical team and data entry personnel, who will ensure that they are accurate and complete. Adverse events must be recorded on the appropriate electronic case report form, assessed for severity and relationship, and reviewed by the site principal investigator or appropriate sub-investigator.

Data collection is the responsibility of the study personnel at the participating clinical study site under the supervision of the site principal investigator. During the study, the site principal investigator must maintain complete and accurate documentation for the study.

The SDCC for this study will be responsible for data management, quality review, analysis, and reporting of the study data.

13.3 Data Capture Methods

Clinical (including, but not limited to, AE/SAEs, concomitant medications, medical history, physical assessments, and clinical laboratory values), reactogenicity and immunogenicity data will be entered into eCRFs via a 21 CFR Part 11-compliant internet data entry system provided by the DCC. The data system includes password protection and internal quality checks, such as

automatic range checks, to identify data that appear inconsistent, incomplete, or inaccurate. Clinical data will be entered directly into electronic case report forms by the study personnel.

13.4 Types of Data

Data for this trial will include clinical, safety, and outcome measures (e.g., clinical assessments, clinical laboratory values, solicited events, and immunogenicity data).

13.5 Study Records Retention

Study records and reports including, but not limited to, eCRFs, source documents, ICFs, laboratory test results, and study drug disposition records will be retained for 2 years after a marketing application is approved for the study product for the indication for which it is being investigated; or, if no application is to be filed or if the application is not approved for the study product, until 2 years after the investigation is discontinued and the FDA has been notified. These documents will be retained for a longer period, however, if required by local regulations. ICFs for future use will be maintained as long as the sample/specimen exists.

No records will be destroyed without the written consent of the sponsor. It is the responsibility of the sponsor to inform the site principal investigator when these documents no longer need to be retained. The participating VTEU sites must contact DMID for authorization prior to the destruction of any study records.

14 CLINICAL MONITORING

Site monitoring is conducted to ensure that the human subjects' protections, study and laboratory procedures, study intervention administration, and data collection processes are of high quality and meet sponsor, ICH/GCP guidelines and applicable regulations, and that this trial is conducted in accordance with the protocol, protocol-specific MOP and applicable sponsor standard operating procedures (SOPs). DMID, the sponsoring agency, or its designee will conduct site-monitoring visits as detailed in the clinical monitoring plan.

Site visits will be made at standard intervals as defined by DMID and may be made more frequently as directed by DMID. Monitoring visits will include, but are not limited to, review of regulatory files, accountability records, eCRFs, informed consent forms, medical and laboratory reports, and protocol and GCP compliance. Site monitors will have access to each participating site, study personnel, and all study documentation according to the DMID-approved site monitoring plan. Study monitors will meet with site principal investigators to discuss any problems and actions to be taken and will document site visit findings and discussions.

15 PUBLICATION POLICY

Following completion of the study, the lead Principal Investigator is expected to publish the results of this research in a scientific journal. All investigators funded by the NIH must submit or have submitted for them to the National Library of Medicine's PubMed Central (http://www.ncbi.nlm.nih.gov/pmc/) an electronic version of their final, peer-reviewed manuscripts upon acceptance for publication, to be made publicly available no later than 12 months after the official date of publication. The NIH Public Access Policy ensures the public has access to the published results of NIH funded research. It requires investigators to submit final peer-reviewed journal manuscripts that arise from NIH funds to the digital archive PubMed Central upon acceptance for publication. Further, the policy stipulates that these papers must be accessible to the public on PubMed Central no later than 12 months after publication.

Refer to:

- NIH Public Access Policy, http://publicaccess.nih.gov/
- NIH Office of Extramural Research (OER) Grants and Funding, http://grants.nih.gov/grants/oer.htm

As of January 2018, all clinical trials supported by the NIH must be registered on ClinicalTrials.gov, no later than 21 days after the enrollment of the first subject. Results of all clinical trials supported by the NIH, generally, need to be submitted no later than 12 months following the primary completion date. A delay of up to 2 years is available for trials that meet certain criteria and have applied for certification of delayed posting.

As part of the result posting a copy of this protocol (and its amendments) and a copy of the Statistical Analysis Plan will be posted on ClincialTrials.gov.

For this trial the responsible party is DMID which will register the trial and post results.

The responsible party DMID to request certification of delayed posting.

Refer to:

- Public Law 110-85, Section 801, Clinical Trial Databases
- 42CFR11
- NIH NOT-OD-16-149

16 LITERATURE REFERENCES

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17 APPENDICES

Appendix A*: Schedule of Events*

Appendix B*: Toxicity Table/Laboratory Adverse Event Grading Scales*

Appendix C*: Vital Signs ADVERSE Event Grading Scale*

Appendix D*: Diarrhea/Vomiting Grading Scale*

Appendix E*: Solicited Adverse Events Grading Scale*

Appendix F*: Modified Vesikari Score*

Appendix A: Schedule of Events

	Outpatient Screening		Inpatient Challenge Admission					Outpatient Follow-Up					Final Study Contact
Study Day	Day -60 to Day -2 Visit 00A [$]	Day -1 Visit 00B[$]	Day 1 Visit 01	Day 2 Visit 02	Day 3 Visit 03	Day 4 Visit 04	Day 5* Visit 05	Day 6+2 clinic visit Visit 06	Day 15+/-1 clinic visit Visit 07	Day 30 -2/+5 clinic visit Visit 08	Day 45+/-5 clinic visit Visit 09	Day 60+/-5 clinic visit Visit 10	Day 180+/-14 study contact visit Visit 11
Informed Consent	X	(X)	(X)										
Test Of Understanding[!]	X	(X)	(X)										
Medical History/ (Demographics at screening)	X	X	(X)					X	X	X	X	X	X
Concomitant Medication History[1] or Review	X	X	(X)	X	X	X	X	X	X	X			
I/E Criteria Review	X	X	X										
Physical Examination (Abbreviated) [#]	X												
Clinical Assessment/Symptom Directed PE[2]		X	X	X	X	X	X	(X)	(X)	(X)	(X)	(X)	
Monitor/Record Fluid Input/Output (I/Os) [=]				X	X	X	X						
Vital Signs[3,4]	X	X	X[4]	X	X	X	X	X	(X)	(X)	(X)	(X)	
Body Weight[5]	X	X	(X)	(X)	(X)	(X)	(X)						
Chemistry[6, 8]	X		(X)	(X)	(X)	(X)	(X)						
Hematology[7, 8]	X		(X)	(X)	(X)	(X)	(X)						
Urinalysis[9]	X		(X)	(X)	(X)	(X)	(X)						
Urine opiate screen	X												
Urine or serum Pregnancy Test (females of childbearing potential)	X	X	(X)										

	Outpatient Screening		Inpatient Challenge Admission					Outpatient Follow-Up					Final Study Contact
Study Day	Day -60 to Day -2 Visit 00A [$]	Day -1 Visit 00B[$]	Day 1 Visit 01	Day 2 Visit 02	Day 3 Visit 03	Day 4 Visit 04	Day 5* Visit 05	Day 6+2 clinic visit Visit 06	Day 15+/-1 clinic visit Visit 07	Day 30 - 2/+5 clinic visit Visit 08	Day 45+/-5 clinic visit Visit 09	Day 60+/-5 clinic visit Visit 10	Day 180+/-14 study contact visit Visit 11
HIV, HepB surface Ag, Hep C Ab	X												
COVID-19 antigen test		X											
Serum norovirus antibody (IgG, and IgA ELISA and blocking antibody)		X	(X)						X	X			
RNAseq assay (PAXgene) Future Use		X	(X)	X		X		X	X	X			
Blood for cytokine by Luminex assays (plasma) Future Use		X	(X)	X		X		X	X				
Blood for DC/NK (PBMC) Future Use		X	(X)	X		X		X	X				
Norovirus GII.4 -specific Memory B cells/ELISpot assay (PBMC)		X	(X)							X		X	
Norovirus GII.4 -specific T cell/intracellular cytokine assay (PBMC) Future Use		X	(X)					X	X	X			
Norovirus GII.4 -specific ASC ELISpot and ALS ELISA		X	(X)					X	X				
Saliva for secretor status [10] (phenotype)	X												
Stool collection system ("stool kit") and instructions provided		X	X	X	X	X	X	X	X	X	X		

	Outpatient Screening		Inpatient Challenge Admission					Outpatient Follow-Up					Final Study Contact
Study Day	Day -60 to Day -2 Visit 00A [$]	Day -1 Visit 00B[$]	Day 1 Visit 01	Day 2 Visit 02	Day 3 Visit 03	Day 4 Visit 04	Day 5* Visit 05	Day 6+2 clinic visit Visit 06	Day 15+/-1 clinic visit Visit 07	Day 30 - 2/+5 clinic visit Visit 08	Day 45+/-5 clinic visit Visit 09	Day 60+/-5 clinic visit Visit 10	Day 180+/-14 study contact visit Visit 11
Fecal IgA (Future Use) *** ****		X	(X)	X		X		X	X	X			
Stool Collection for shedding of norovirus PCR[11, 12 □]		X	X	X	X	X	X	X	X	X	X	X[□]	
Stool Collection for microbiomes (Future Use)[12****]		X	(X)	X		X		X	X	X			
Stool Collection for cytokines (Future Use)[12]		X	(X)	X		X		X	X	X			
Challenge Administration[13]			X										
Solicited Adverse Events Assessments			X	X	X	X	X						
Illness/Solicited Adverse Events Memory Aids[14^]							X^	X^	X^	X^			
Adverse Event Monitoring[15]			X	X	X	X	X	X	X	X	X	X	X
Discharge from Inpatient Facility (if discharge criteria met)							X[+]						

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1. On Day 1, confirm that the subject has not taken any prohibited medications prior to challenge. All concomitant medications taken in the 30 days prior to study enrollment through Day 30 or early termination will be recorded.

2. Symptom directed physical examination.

3. Vital Signs include blood pressure, pulse, and oral temperature. They will be taken daily during the inpatient challenge unless otherwise specified in the protocol or if medically indicated.

4. Vital signs will be obtained prior to challenge administration on Day 1 and approximately 30 and 60 minutes after receipt of the challenge virus, and more frequently if medically indicated.

5. Weight will be checked at screen and baseline, prior to challenge. Weight will be obtained at other times as clinically indicated.
6. Serum chemistry parameters include alanine transaminase (ALT), total bilirubin, creatinine, potassium, sodium.
7. Hematology parameters include white blood cells (WBC) with neutrophils (ANC), hemoglobin, and platelet count.
8. Screening labs (chemistry and hematology) will be repeated if original labs obtained more than 60 days before challenge. Subjects who receive IV hydration during the inpatient admission will have safety labs collected, if indicated per investigator discretion and will be required to have clinically acceptable electrolyte values for sodium and potassium prior to discharge.
9. Urinalysis parameters include urine protein. At the discretion of the investigator or delegate subjects may have a urine specific gravity performed to capture urine results in real time.
10. Saliva for secretor status at screening. If available, results from previous testing for secretor status may be used for screen and need not be repeated.
11. A rectal swab may be used for detection of Norovirus shedding, if a bulk stool cannot be provided.
12. One stool sample will be collected on Day -1 or Day 1 baseline, prior to challenge. If additional stool is produced by a subject after the initial sample but prior to challenge, the sample will not be collected nor entered to Global Trace. After challenge, stools will be collected as described in the MOP. At least 2 aliquots from each stool collected while inpatient will be processed for norovirus shedding. Samples also will be collected and stored for microbiome testing. Stool samples for microbiome testing, cytokine detection, and fecal IgA must be bulk samples.
13. Subjects may have clear liquids within 4 hours of challenge but will be NPO (nothing by mouth) for 90 minutes before and for 90 minutes after challenge administration.
14. Subjects will complete a daily memory aid of illness/solicited events symptoms, changes in medical status, and concomitant medications. Subject's memory aids will be reviewed at the Day 6 visit. Study staff will attempt to collect and retain the illness/solicited events memory aid at the Day 15 visit.
15. Adverse events will be collected to Day 30. Adverse events limited to new-onset chronic medical conditions and Serious Adverse Events will be collected up to Day 180.

(X) Represents study related procedures that are not required but *may* be done on this day, if indicated. (i.e. Subjects may be admitted the day before challenge or on the day of challenge. Some study related procedures may occur within 24 hours of challenge.).

* Subjects needing to remain past Day 5 will have study evaluations repeated daily until discharge.

*** Efforts will be made to collect 2 baseline bulk samples prior to challenge, including morning of challenge.

****Since fecal IgA stools will be sent to the Fisher Repository, and then microbiome samples can be sent to the repository as well. Samples may be collected together (with appropriate vials) as microbiome and fecal IgA samples for future use with appropriate collection days.

▫ Subjects who continue to shed Norovirus at the Day 60 visit will be followed for clearance.

!! Subjects will be provided with educational materials at screening and given complete education about study participation. A sample test may be given during the education process. If a subject does not score a 70% on the exam, he/she may re-take the test.

An abbreviated physical examination is distinguished from a complete physical exam in that all assessments are not required (e.g. genito-urinary and rectal exams).

= All emesis will be measured. All stools will be graded for consistency (see Appendix D: Diarrhea/Vomiting Grading Scale for details), and all loose or watery stools (i.e., those that conform to the shape of the container) also will be weighed. Subjects who develop diarrhea will be asked to drink 1.5 mL of ORS for each gram of diarrheal stool that they produce. Subjects developing vomiting will be asked to drink a volume of ORS equivalent to the amount of the emesis.

$ Screening evaluations may occur over 1 or more visits.

+ Subjects will be provided instructions and “stool kit” to collect sample.

@ Provide subject with digital thermometer and instructions in its use and asked to take and record their oral temperature once daily at the same time each day for 5 days after discharge.

^ Remind/review with subjects to contact the site if a household contact or anyone they have daily contact with develops symptoms of Norovirus infection within a week after being discharged from the inpatient unit.

Appendix B: Toxicity Table/Laboratory Adverse Event Grading Scale

Adverse Event Grading

This protocol is CDISC compliant. During screening, subjects will have blood drawn to determine if any clinical laboratory abnormalities exist that would preclude study participation.

Laboratory	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)
Sodium, low, mEq/L	132 – 135	130 – 131	125 – 129
Sodium, high, mEq/L	146 – 147	148 – 149	150 or greater
Potassium, high, mEq/L	5.2 – 5.3	5.4 – 5.5	5.6 – 5.7
Potassium, low, mEq/L	3.3 – 3.4	3.1 – 3.2	2.9 – 3.0
Creatinine, high mg/dL (female)	1.0 - 1.1	1.2 - 2.0	2.1 – 2.5
Creatinine, high mg/dL (male)	1.2 – 1.3	1.4 – 2.0	2.1 – 2.5
Liver Function Tests (ALT) increase by factor	>1.1-2.5 x ULN	>2.5-5.0 x ULN	>5.0x ULN
Total bilirubin	>1.0-1.5 x ULN	>1.5-2.0 x ULN	>2.0 x ULN
Hgb (female), low g/dL	10.9-11.6	9.4 – 10.8	≤9.3
Hgb (male), low g/dL	13.1-13.2	12.5-13.0	≤12.4
WBC, increase, cells, x 10^3u/L	>11.1 –≤15.0	>15 - ≤ 20	>20.0
WBC, decrease, cells, x 10^3u/L	2.5-<4.4	1.5-<2.5	<1.5
Absolute Neutrophil Count (ANC), x 10^3u/L	1.2-<1.7	0.9-<1.2	<0.9
Platelets, decrease, cells/mm^3	124,000-<134,000	100,000<124,000	<100,000
Urine protein	Trace	1+	2+

Appendix C: Vital Signs Adverse Event Grading Scale

Vital Signs Grading		
Adverse Event	**Severity**	**Parameter**
Fever	1	38.0 – 38.4°C 100.4 – 101.1°F
	2	38.5 – 38.9°C 101.2 – 102.0°F
	3	>38.9°C >102°F or ER visit or hospitalization
Hypertension (systolic)}	1	151-155 mm Hg
	2	156-160 mm Hg
	3	>160mm Hg or ER visit or hospitalization
Hypertension (diastolic)	1	91 – 95 mm Hg
	2	96-100 mm Hg
	3	>100 mm Hg or ER visit or hospitalization
Hypotension (systolic)	1	85-89 mm Hg
	2	80-84 mm Hg
	3	<80 mm Hg or ER visit or hospitalization
Bradycardia*	1	45-50bpm *
	2	40-44 bpm
	3	<40 bpm or ER visit or hospitalization
Tachycardia	1	101-115 bpm
	2	116-130 bpm
	3	>130 bpm or ER visit or hospitalization

* If subject baseline heart rate is <45 beats per minute and the investigator determines that this is not clinically significant (e.g., athletes) and heart rate increases >45 beats per minute on moderate exercise (two flights of stairs), this will not be considered a grade 1 adverse event. *If a subject has significant abnormalities in their heart rate, they will be informed of the values and advised to seek care from their physician.*

} *If a subject has significant abnormalities in their blood pressure, they will be informed of the values and advised to seek care from their physician.*

Appendix D: Diarrhea/Vomiting Grading Scale

Stool consistency will be determined according to the guide below:

Stool Descriptor	Definitions
Normal	Firm, tootsie roll consistency, soft pudding consistency
Loose	Runny, takes the shape of the container, gravy consistency, brown liquid, opaque liquid, chocolate milk consistency
Liquid/Watery	Clear liquid, rice water, soapy water consistency

All stools will be graded according to the Stool Grading Guide below:

Clinical Feature	Grade 0	Grade 1	Grade 2	Grade 3
Diarrhea	None	In a 24/h period: 3 loose stools or liquid stools **or** 300-599 gm of loose or liquid stool	In a 24/h period: 4-5 loose stools or liquid stools **or** 600-800 gm of loose or liquid stool	In a 24/h period: ≥6 liquid stools **or** >800 gm of loose or liquid stool
Vomiting	None	1-2 episodes in 24 hours	3-5 episodes in 24 hours	>5 episodes in 24 hours

Note: -2 loose or liquid stools or <300gm stool in a 24h period are not diarrhea,

Appendix E: Solicited Adverse Events Grading Scale

Clinical Feature	Grade 0	Grade 1 (mild)	Grade 2 (moderate)	Grade 3 (severe)
Fever (oral temperature)	none	100.4 – 101.1°F (38.0 – 38.4°C)	101.2 – 102.0°F (38.5 – 38.9°C)	>102^0F (>38.9°C) or ER visit or hospitalization
Headache	none	no pain medications taken	use of pain medication required	narcotic pain med required and/or prevents daily activity
Nausea	None	mild*	moderate**	severe***
Abdominal Cramps/discomfort/ pain	none	mild*	moderate**	severe***
Abdominal gurgling	none	mild*	moderate**	severe***
Abdominal bloating	none	mild*	moderate**	severe***
Myalgia	none	mild*	moderate**	severe***
Malaise, fatigue	none	mild*	moderate**	severe***
Anorexia, loss of appetite	none	mild*	moderate**	severe***
Chills	none	mild*	moderate**	severe***

Grading Scale

0 = None

1 = *Mild, No interference with activity

2 = **Moderate, Some interference with activity

3 = ***Severe, Significant interference, prevents daily activity

Appendix F: Modified Vesikari Score

Modified Vesikari Scale (17 Point Scale)	
Duration of Diarrhea (days)	**Points**
1	1
2-3	2
≥4	3
Maximum Number of Diarrheal Stools/ 24 hours	
3	1
4-5	2
≥6	3
Duration of Vomiting (days)	
1	1
2	2
≥3	3
Maximum Number of Vomiting Episodes/ 24 hours	
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