A Phase 2 Multi-Center, Prospective, Randomized, Double-Blind Study to Assess the Clinical and Antiviral Efficacy and Safety of Nitazoxanide for the Treatment of Norovirus in Hematopoietic Stem Cell and Solid Organ Transplant Recipients

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21 January, 2020

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

All key personnel (all individuals responsible for the design and conduct of this study) have completed Human Subjects Protection Training.

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: _____

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LIST OF ABBREVIATIONS

AE	Adverse Event/Adverse Experience
AIDS	Acquired Immune Deficiency Syndrome
ALC	Absolute Lymphocyte Count
BID	Twice Daily
CBC	Complete Blood Count
CFR	Code of Federal Regulations
CKD	Chronic Kidney Disease
COR	Contracting Officer's Representative
CRF	Case Report Form
DHHS	Department of Health and Human Services
DMID	Division of Microbiology and Infectious Diseases, NIAID, NIH, DHHS
DSMB	Data and Safety Monitoring Board
eCRF	Electronic Case Report Form
EIA	Enzyme immunoassay
FDA	Food and Drug Administration
FWA	Federal Wide Assurance
GCP	Good Clinical Practice
GVHD	Graft Versus Host Disease
HIPAA	Health Insurance Portability and Accountability Act
HIV	Human Immunodeficiency Virus
HSCT	Hematopoietic Stem Cell Transplantation
IB	Investigator's Brochure
ICF	Informed Consent Form
ICH	International Conference on Harmonization
ICMJE	International Committee of Medical Journal Editors
ICU	Intensive Care Unit
IND	Investigational New Drug Application
IRB	Institutional Review Board
JAMA	Journal of the American Medical Association
MOP	Manual of Procedures
N	Number (typically refers to subjects)
NEJM	New England Journal of Medicine
NIAID	National Institute of Allergy and Infectious Diseases
NIH	National Institutes of Health
NUCTC	Northwestern University Comprehensive Transplant Center
OCRA	Office of Clinical Research Affairs, DMID, NIAID, NIH, DHHS

OHRP	Office for Human Research Protections
OHSR	Office for Human Subjects Research
ORA	Office of Regulatory Affairs, DMID, NIAID, NIH, DHHS
PHI	Protected Health Information
PI	Principal Investigator
PK	Pharmacokinetics
PRO	Patient Reported Outcomes
QA	Quality Assurance
QC	Quality Control
QOL	Quality of Life
RT-PCR	Reverse Transcription Polymerase Chain Reaction
SAE	Serious Adverse Event/Serious Adverse Experience
SDCC	Statistical Data and Coordinating Center
SOP	Standard Operating Procedure
SOT	Solid Organ Transplant
UGI	Upper Gastro Intestinal
UNOS	United Network for Organ Sharing
US	United States
WHO	World Health Organization

PROTOCOL SUMMARY

Title:	A Phase 2 Multi-Center, Prospective, Randomized, Double-Blind Study to Assess the Clinical and Antiviral Efficacy and Safety of Nitazoxanide for the Treatment of Norovirus in Hematopoietic Stem Cell and Solid Organ Transplant Recipients
Phase:	Phase 2
Population:	**Sample Size:** 160 male or female Hematopoietic Stem Cell or Solid Organ transplant recipients, equal to or greater than 12 years of age with diagnosis of Norovirus.
Number of Sites:	12
Study Duration:	Approximately 60 months
Subject Participation Duration:	Approximately 6 months
Description of Agent or Intervention:	1. Nitazoxanide Arm: All subjects age ≥ 12 years: 500 mg (one tablet) Nitazoxanide by mouth twice daily with food for 56 consecutive doses. 2. Placebo Arm: All subjects age ≥ 12 years: Placebo (one tablet) by mouth twice daily with food for 56 consecutive doses
Objectives:	**Primary Objective:** 1. To assess the *clinical efficacy* of nitazoxanide for the management of acute and chronic Norovirus in transplant recipients. **Secondary Objectives:** 1. To assess the *virologic efficacy* of nitazoxanide. 2. To assess the *safety* of nitazoxanide for the management of acute and chronic Norovirus in transplant recipients. **Exploratory Objectives:** 1. To assess initial *clinical improvement* in Norovirus Disease through 28 and 180 days.

2. To assess *markers of virologic improvement* of Norovirus Disease through 28 and180 days.
3. To assess the *contribution of immunologic response* to Norovirus to the clinical course of disease in treated and untreated subjects.
4. To define the *pharmacokinetics and dose response relationships* of nitazoxanide.
5. To define the *natural history* of Norovirus in nitazoxanide treated and untreated subjects.

Outcome Measures

Primary Outcome:

1. Time from randomization until initial clinical resolution of Norovirus symptoms for at least 48 hours through Day 180. Clinical resolution will be assessed from the daily diary by the subject and will be defined as cessation of vomiting and no stools classified by the Bristol Stool Chart as diarrhea (Type 6 or 7).

Secondary Outcomes:

Virologic Efficacy

1. Time from the randomization to first negative viral load through 180 days.
2. The change in viral titer between Day 1 and 180.

Safety

3. Incidence of unsolicited non-serious adverse events (see section 9 for details) through 60 days.
4. Incidence of laboratory adverse events (WBC, Hemoglobin, Platelet count, Creatinine, Alkaline Phosphatase, ALT, AST, BUN and Bilirubin) through 60 days.
5. Incidence of protocol-specified serious adverse events (see section 9 for details) through 60 days.
6. Incidence of Hospitalization through 60 days.

Exploratory Outcomes:

Clinical Improvement:

1. Time from randomization to initial clinical improvement in Norovirus disease through 28 and 180 days for at least 48 hours, as defined by:
    a. 50% reduction in number of episodes of vomiting and,

   b. Improvement in PO intake, as judged by the subjects and,
   c. 50% reduction in the number of episodes of diarrhea as classified by the subject by the Bristol Stool Chart as type 6 or 7 bowel movements,
2. Time from randomization to initial cessation of vomiting through Day 180 (recurrent vomiting will be considered disease recurrence unless determined to be due to another etiology [i.e. GVHD]).
3. Time from randomization to subject report of no loss of appetite that is maintained for 48 hours through Day180 (measure will be first time that the subject reports that they have no "loss of appetite" on their daily diary).
4. Time from randomization to initial cessation of diarrhea through Day 180 classified by the Bristol Stool Chart as type 6 or 7 bowel movements (recurrent diarrhea will be considered disease recurrence unless determined to be due to another etiology [i.e. GVHD]).
5. Time from randomization to 50% reduction in the amount of antimotility agents utilized by the subject through Day 180.
6. Number of days of diarrhea through Day 28 and 180.
7. Number of stools classified by the Bristol Stool Chart as type 6 or 7 bowel movements through Day 28.
8. Number of days of IV hydration or total parenteral nutrition (TPN) therapy through Day 28.
9. Total number of days of hospitalization through Day 28 and 180.
10. Change in patient-reported quality of life as measured by EuroQOL-5 (global) and IBSQOL (diarrhea specific) [adult] or Euro QOL-5 Peds (global) and PedsQL GI Module PedsQL [children] from baseline to Day 180.
11. Change in patient-reported physical function as measured by the NIH PROMIS Physical Function (Adult) Pediatric Global Health (Peds) from baseline to Day 180.
12. Change in patient-reported emotional distress as measured by the NIH PROMIS Depression, Anxiety, Fatigue (Adult and Peds) from baseline to Day 180.

13. Change in patient-reported gastrointestinal symptoms as measured by the NIH PROMIS GI (Adult and Peds) symptoms (6 of 8 subscales) from baseline to Day 180.
14. The effect of co-pathogens, as determined by the BioFire stool pathogen screen, on the primary outcome measure.
15. Time from randomization until initial clinical resolution of Norovirus symptoms for at least 48 hours through Day 180. Clinical resolution will be assessed from the daily diary by the subject and will be defined as cessation of vomiting and no stools classified by the Bristol Stool Chart as diarrhea (Type 6 or 7) from the total population compared to the subgroup of subjects who do not report chromaturia (discoloration of urine) based on unsolicited self-reporting.
16. Incidence of unsolicited non-serious adverse events (see section 9 for details) through 60 days, laboratory adverse events (WBC, Hemoglobin, Platelet count, Creatinine, Alkaline Phosphatase, ALT, AST, BUN and Bilirubin) through 60 days, protocol-specified serious adverse events (see section 9 for details) through 60 days, and Hospitalization through 60 days from the total population compared to the subgroup of subjects who do not report chromaturia (discoloration of urine) based on unsolicited self-reporting.

Virologic Improvement:

17. Time to ≥ 1 log reduction in stool Norovirus genome copies.
18. Change in quantitative Norovirus load in the stool between Day 1 and Days 7, 14, 21, 28, 60, 120, and 180.
19. Change in Norovirus sequence from Day 1 to Days 28 and 60.
20. The effect of co-pathogens, as determined by the BioFire stool pathogen screen, on the time from the randomization to first negative viral load through 180 days.
21. Time from the randomization to first negative viral load through 180 days and the change in viral titer between Day 1 and 180 from the total population compared to the subgroup of subjects who do not report chromaturia (discoloration of urine) based on unsolicited self-reporting.

Immunologic Response:

22. Change in Norovirus-specific serum and stool IgA, IgM and IgG between Day 1 and Days 28 and 60 (stool only).
23. Association between change in total lymphocyte count and T cell subsets at Day 1 and 180 and clinical resolution of Norovirus symptoms.
24. Association between change in total lymphocyte count and T cell subsets at Day 1 and 180 and undetectable quantitative Norovirus PCR.
25. Association between change in Norovirus-specific serum and stool IgA, IgM and IgG from Day 1 to Days 28 and 60 (stool only) and clinical resolution of symptoms.
26. Association between change in Norovirus-specific serum and stool IgA, IgM and IgG from Day 1 to Days 28 and 60 (stool only) and undetectable quantitative Norovirus PCR.

Pharmacokinetics and Dose Response:

27. For subjects consenting to PK draws, concentrations of nitazoxanide metabolites (tizoxanide and tizoxanide glucuronide) 1 to 4 hours after the first dose on Day 7 and 10 minutes before the second dose on Day 21.

Natural History of Disease

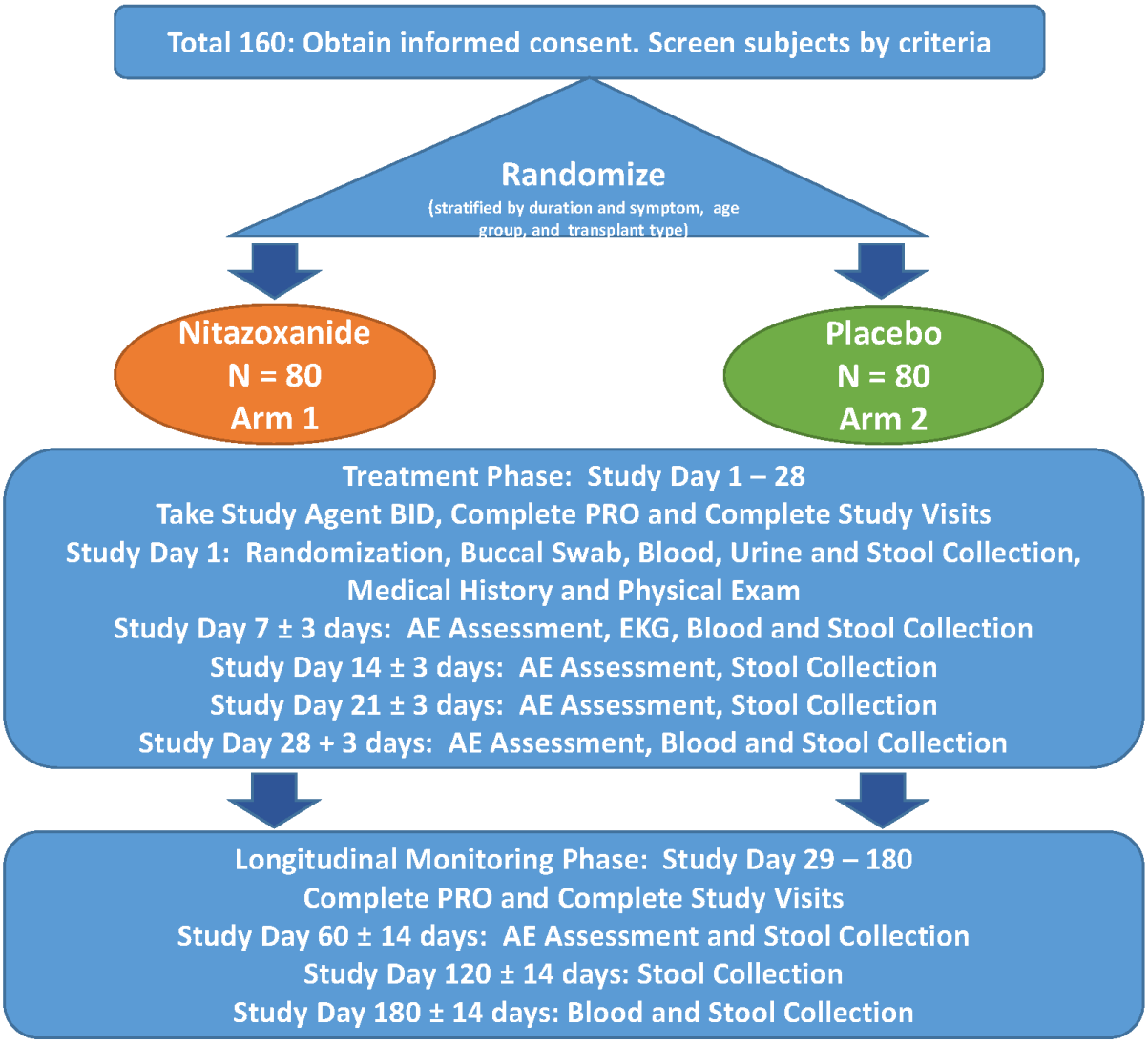
28. Time to Allograft rejection as per each center team.
29. Time to allograft loss as reported to UNOS.
30. Time to death.
31. Time to withdrawal from the study because of intolerance or due to drug-related adverse events.
32. Correlation of secretor status as defined by phenotype and genotype, separately, and clinical resolution of Norovirus as defined by the primary endpoint.

Description of Study Design:

A total of 160 subjects ≥ 12 years of age will be selected according to the inclusion and exclusion criteria. Upon enrollment in the study, subjects will be randomized into two treatment groups: active nitazoxanide and placebo. Randomization will be stratified by age group (pediatric (12-17

	years) vs. adult ≥ 18 years), chronicity of Norovirus-associated symptoms (acute (<14 days) vs. chronic (≥14 days)) and transplant type (solid organ (SOT) vs. hematopoietic stem cell transplant (HSCT)). Enrolled subjects will participate in 2 phases of the study: Treatment Phase, which will include dosing with the assigned study agent for 28 days and study visits on study Day 1, 7 ± 3 days, 14 ± 3 days, 21 ± 3 days, and 28 + 3 days; Longitudinal Monitoring Phase which will include telephone call on Days 35 ± 3 days, 53 ± 7 days, 113 ± 7 days, 173 ± 7 days. study visits on study Day 60 ± 14 days, 120 ± 14 days and 180 ± 14 days. If symptoms recur after completion of the Treatment Phase, subjects can resume any therapy excluding nitazoxanide at the discretion of the principal investigator or his/her designee. Nitazoxanide can be used after Day 28 if the local PI attests that it is medically urgent to use the nitazoxanide because the patient is experiencing severe or life-threatening disease. Although nitazoxanide can be used if it is felt critical to the subjects care, its use should be avoided until Day 180 whenever felt to be safe to do so by the local PI. Additional stool specimens should be collected prior to initiation of therapy for testing in the central lab.
Estimated Time to Complete Enrollment:	60 months.

Schematic of Study Design:


Total 160: Obtain informed consent. Screen subjects by criteria
Randomize
(stratified by duration and symptom, age group, and transplant type)
Nitazoxanide
N = 80
Arm 1
Placebo
N = 80
Arm 2
Treatment Phase: Study Day 1 – 28
Take Study Agent BID, Complete PRO and Complete Study Visits
Study Day 1: Randomization, Buccal Swab, Blood, Urine and Stool Collection, Medical History and Physical Exam
Study Day 7 ± 3 days: AE Assessment, EKG, Blood and Stool Collection
Study Day 14 ± 3 days: AE Assessment, Stool Collection
Study Day 21 ± 3 days: AE Assessment, Stool Collection
Study Day 28 + 3 days: AE Assessment, Blood and Stool Collection
Longitudinal Monitoring Phase: Study Day 29 – 180
Complete PRO and Complete Study Visits
Study Day 60 ± 14 days: AE Assessment and Stool Collection
Study Day 120 ± 14 days: Stool Collection
Study Day 180 ± 14 days: Blood and Stool Collection

1 KEY ROLES

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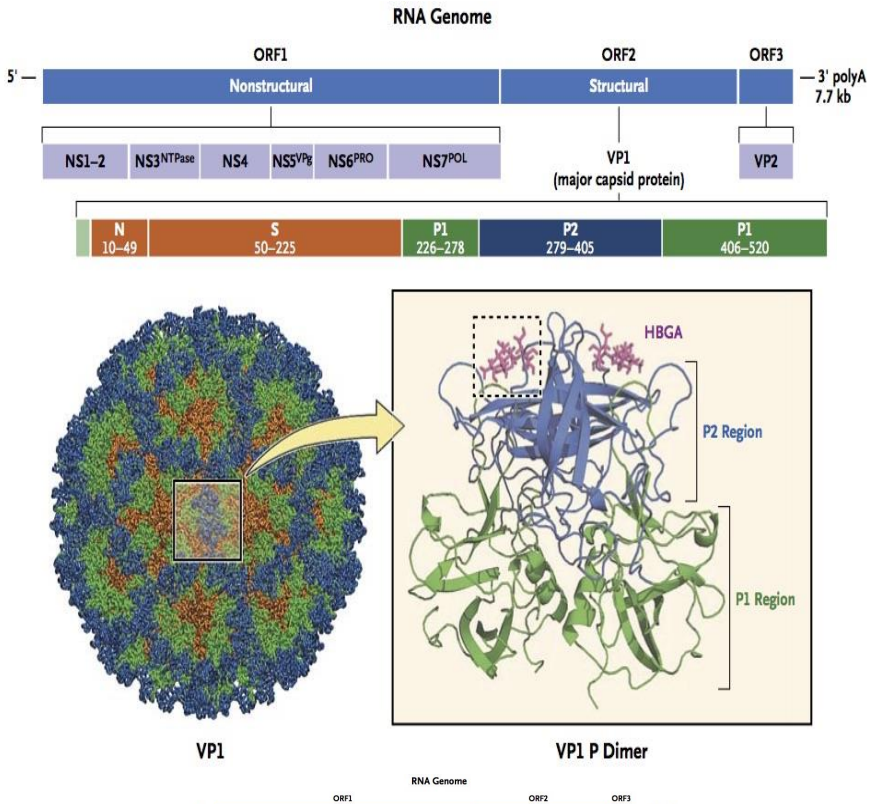
2 BACKGROUND INFORMATION AND SCIENTIFIC RATIONALE

2.1 Background Information

Norovirus

Gastroenteritis is defined as a self-limiting diarrheal illness that is often accompanied by nausea, vomiting, fever, or abdominal pain. In the United States, Norovirus is the single most common cause of acute gastroenteritis that leads to medical evaluation in adults, and the second most common cause of severe diarrhea in infants and young children.[3, 5] In the US, Norovirus is estimated to be responsible for 19–21 million episodes of gastroenteritis and 56,000–71,000 hospitalizations annually.[1] Increasingly, Norovirus is recognized as a common cause of chronic gastroenteritis in immunocompromised patients. Prolonged diarrhea can lead to dehydration, allograft dysfunction, renal insufficiency and rarely death in these patients.[3, 6-11]

Figure 1:Norovirus Capsid & Genome (3)



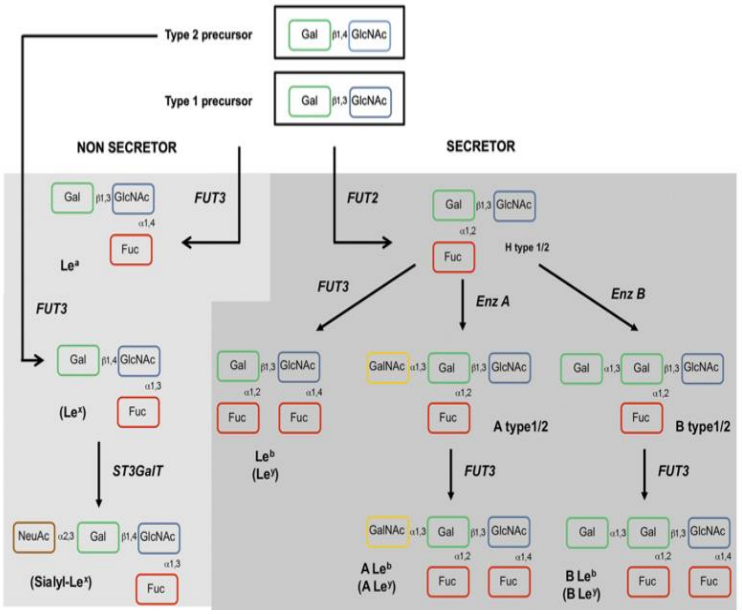
Noroviruses are small, non-enveloped, single-stranded RNA viruses that are members of the Caliciviridae (Figure 1). [3, 12] Of the five genogroups (GI-GV), GI, GII and GIV are known to be human pathogens, although most are caused by GI and GII, which can be further classified into 30 unique genotypes. GII.4 has been recognized as the most common cause of outbreaks of Norovirus infections globally. [13, 14]

Norovirus is highly contagious as transmission is highly efficient with a median infectious dose of 18 viruses. There is generally high titers of virus within stool (10^5 to 10^{10} genome copies per gram of feces); [15, 16]

therefore contact with infected feces and vomit, fecally-contaminated food, water and surfaces are common sources of infection.[17, 18, 15, 16] Norovirus is also highly stable with retained infectivity under environmental conditions that would generally inactivate other viruses, including chlorine at concentrations used in drinking water.[19, 20]

The Norovirus genome encodes 2 major structural proteins (VP1 and VP2) and 7 nonstructural proteins responsible for viral replication. The viral VP1 protruding 2 (P2) domain binds saccharides of the human ABO, secretor (H), and Lewis families histo-blood group antigens to facilitate viral entry into the epithelial cells of the gastrointestinal tract.[4, 20, 21] Carbohydrate antigen expression on gastrointestinal epithelial cells are controlled by enzymes encoded by the ABO, FUT2 and FUT3 alleles in humans. Only 70-80% of individuals have a functional copy of the *FUT2* ("secretor") gene required for gut HBGA expression (Figure 2); these individuals are known as "secretors." "Non-secretors" have inactivated FUT2 alleles typically due to homozygous G428A nonsense mutation or A385T missense mutation. Non-secretors appear to have reduced susceptibility or outright resistance to symptomatic infection.[22-25] Further, *in vitro* work suggests that A and B blood group blood type B patients have reduced susceptibility to infection.[26] However, it remains unclear if these proteins serve only as attachment ligands on host cells or are functional receptors that enable entry into the cells.

Figure 2: HBGA Biosynthesis (4)



While significant progress has been made in understanding host factors that modulate the risk of developing Norovirus disease, our understanding of the immune response to Noroviruses remains limited.[20] Adaptive immunity appears to play an incomplete role in the prevention and control of Norovirus infections. There appears to be short and long-term immunity to infection based on data from challenge studies. Re-challenge with the same serotype within 6-14 weeks of initial infection generally prevents recurrent infections but not infection with heterotypic viruses.[27] Long-term immunity is more complex and re-challenge with the same virus 27-42

months results in variable resistance that does not correlate with seroconversion. In fact, data suggests that patients without pre-challenge antibodies are less likely to develop symptoms. Local mucosal IgA responses appear to be protective. More recently, HBGA carbohydrate-blocking antibodies that block Norovirus VLPs to H type 1 or H type 3 glycans appear to be protective.[20, 28] Cell-mediated responses, including both T- and B-cell responses, are critical for clearance of Norovirus. Following challenge with VLPs and Snow Mountain virus, there is a robust T-helper type 1 (Th-1) response with significant increases in interferon-α and IL-2, but not IL-4, IL-6, or IL-10.[29] Depletion of $CD4^+$ cells results in reduced interferon production.[29] In a mouse model, $CD4^+$ and $CD8^+$ cells were required for clearance of murine Norovirus in the intestine.[30] Likewise, patients with defective T cell function more commonly have prolonged shedding and clearance of shedding in patients with chronic infection has been shown to be associated with the recovery of T cells.[31, 32]

Cell-mediated responses, including both T- and B-cell and B-cell responses, are critical for clearance of Norovirus. Following challenge with VLPs and Snow Mountain virus, there is a robust T-helper type 1 (Th-1) response with significant increases in interferon-γ, IL-2 but not IL-4, IL-6, or IL-10.[29] Depletion of $CD4^+$ cells results in reduced interferon production.[29] Further, in a mouse model, $CD4^+$ and $CD8^+$ cells were required for clearance of murine Norovirus in the intestine.[30] Likewise, patients with defective T cell function more commonly have prolonged shedding and clearance of shedding in patients with chronic infection has been shown to be associated with the recovery of T cells.[31, 32] Norovirus appears to evolve rapidly with defects in cellular immunity (3.3% amino acid substitutions per year in chronic shedders) [3].

Norovirus infections typically presents as an acute infection in immunocompetent hosts, with an incubation period of 10 to 51 hours. Symptoms include nausea, vomiting, watery non-bloody diarrhea, abdominal cramps and occasionally low-grade fever, muscle aches, chills and headache,[20] usually lasting from 24 to 60 hours. [33] While the acute phase of illness is typically short-lived, asymptomatic patients may continue to shed the virus for up to two weeks.[12, 20, 34] Unfortunately, approximately 570–800 deaths each year result from Norovirus infections in the US, with one third of fatal cases occurring in immunocompromised hosts.[35] Immunocompromised patients such as those with congenital immunodeficiencies, human immunodeficiency virus (HIV)-infected patients, solid organ transplant recipients, hematopoietic stem cell transplant patients and oncology patients undergoing chemotherapy can develop chronic Norovirus infections. [3, 36-41] Copious watery diarrhea with stool volume amounting to several liters per day comparable to volumes observed with cholera may last for 3–4 months or longer. [1, 37] As a result, dehydration and elevated calcineurin inhibitor levels are common in patients at the time of initial diagnosis.

Chronic Norovirus infection is common in hematopoietic stem cell transplant recipients.[6, 11, 36, 38, 42-45] In one study, 18% of hematopoietic stem cell transplant patients developed Norovirus infection post-transplant, with prolonged shedding detected in most of these patients (median 3 months, range 0.5 – 14 months).[38] Half had severe weight loss as the result of Norovirus gastroenteritis; one patient died of malnutrition attributed to Norovirus infection. In another retrospective study in pediatric hematopoietic stem cell recipients, 16.3% of patients with diarrhea were proven to have Norovirus infections. Norovirus developed at a median of 36.5 days post-transplantation (range, 5-517 days) with prolonged shedding in most patients (median 145, range 13- 263).(11) A more recent study demonstrated prolonged shedding of virus in sera (mean of 33.6 days) and in stool (mean of 61.6 days) in adult HSCT recipients with diarrhea.[44]

Solid organ transplant recipients tend to have prolonged shedding of Norovirus as well.[7, 10, 36, 39, 40, 46-48] One of the earliest studies documented Norovirus in 17% of renal transplant patients who shed virus for 97-898 days. This study was one of the first to sequence Noroviruses and demonstrated accumulation of mutations in several of the patients over time, resulting in amino acid changes predominantly in the P2 and P1-2 region.[40] Recently, our group conducted a retrospective study of solid organ transplant recipients admitted to the hospital with diarrhea. In this study of 422 admissions involving 314 unique patients, Norovirus was the second most common documented cause of diarrhea after *C. difficile* (Table 1).[7] Norovirus was more common among individuals who had community-acquired diarrhea.

Table 1: Cause of Diarrhea in Hospitalized SOT Patients at a Single Center

	Community-Onset Diarrhea (n = 422)		Hospital-Onset Diarrhea (n = 112)		
Etiology	No.	%	No.	%	*P* Value
Single diagnosis (n = 523)					.03
NOS	257	60.9	85	75.9	
Clostridium difficile infection	55	13.0	13	11.6	
Norovirus	34	8.1	3	2.7	
CMV disease/colitis	26	6.2	3	2.7	
Other[a]	42	10.0	6	5.4	

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Other[a]	42	10.0	6	5.4	

Recently, we expanded on our initial study to assess the epidemiology and outcomes of adult

solid organ transplant patients with Norovirus.[49] We retrospectively reviewed data from 67 SOT recipients with diarrhea who tested positive for Norovirus and 67 randomly selected SOT recipients with diarrhea who tested negative for Norovirus. From this data, 35% of all patients evaluated for diarrhea had Norovirus, which was the most common infectious diagnosis among patients with diarrhea; *C. difficile* was the second most common diagnosis in 25% of patients. We found that nearly half of patients with Norovirus were diagnosed without the need for hospitalization. Diarrheal duration was significantly protracted in Norovirus patients with an average of 241 days versus 75 days in the control (p=0.00065); 22.4% of Norovirus patients were still having diarrhea at the time of last evaluation suggesting that we are likely underestimating the duration of symptoms. There were 1 patient death and 3 graft failures related to the Norovirus illness within the first year after the onset of diarrhea and 30% of Norovirus patients had a ≥ 20% increase in creatinine within 1 year of the diagnosis. Most (58%) of the Norovirus-infected patients required intravenous hydration on diagnosis and 81% had supra-therapeutic tacrolimus levels at diagnosis.

In another recent study of 116 pediatric solid organ and hematopoietic stem cell transplant recipients with diarrhea, 22% had documented Norovirus infection, making it the most commonly identified enteropathogen.[50] Over half of these patients had prolonged diarrhea with a median duration of 12.5 days (range 1-324 days) and 29% developed recurrent episode(s) of Norovirus-associated diarrhea. Patients with Norovirus were more likely to require ICU admission (27% vs. 0%, $p = 0.02$), had a greater rise in serum creatinine (median 0.3 vs. 0.2 mg/dL, $p = 0.01$), and experienced greater weight loss (median 1.6 vs. 0.6 kg, $p < 0.01$).

Diagnosis of Norovirus generally relies upon detection of Norovirus RNA or antigens in stool since cell culture is not available and direct visualization via electron microscopy is impractical.[34, 51] Enzyme immunoassay (EIA) detection methods are also available commercially. Although simpler to perform, EIA assays have reduced sensitivity (58–93%) and specificity (85–100%) compared to RT-PCRs.[36, 51] Qualitative or quantitative PCRs can be run on stool, vomitus, foods, and environmental specimens.[34] PCR methods generally use primers directed at highly conserved regions of ORFs 1, 2, and 3, including the POL region in ORF 1.[20, 52] Sequencing the products of RT-PCRs allows tracking of individual strains, which may be useful in outbreaks.[14] Several multiplex panels that detect Norovirus, among other common gastrointestinal pathogens, have recently become widely available.[36]

The current mainstay of therapy for Norovirus is supportive.[53, 54] Reduction of immunosuppression is commonly practiced since diarrhea is a common adverse effect of many immunosuppressive drugs, including mycofenolate mofetil. It remains unclear, however, if reduction of immunosuppression alone significantly improves clearance of Norovirus. There are

a few case reports that suggest that substitution of one of the immunosuppressive agents with one of the mTOR inhibitors may speed recovery, but the strength of this data is limited.[6, 47] In these two cases, symptoms improved within days, and viral shedding was undetectable within weeks of changing to an mTOR inhibitor.

Table 2: Approved and Experimental Therapies with Norovirus Activity

Target	Compound	Assays/stage	Virus
Pol (nucl.)	2′C-methylcytidine	Cell culture (infection)	MNV
		Cell culture (replicon)	NV
		Mouse infection	MNV
Pol (nucl.)	Ribavirin	Cell culture (replicon)	NV
		Cell culture (infection)	FCV
		Co-crystallization w/pol	NV
Pol (nucl.)	2′-F-2′-methylcytidine	Cell culture (infection)	MNV
		Cell culture (replicon)	NV
Pol (nucl.)	B-D-N(4) hydroxycytidine	Cell culture (infection)	MNV
		Cell culture (replicon)	NV
Pol (nucl.)	2-Thio-uridine	Cell culture (infection), RdRp *in vitro* assay	FCV
Pol (nucl.)	6-Aza-uridine	Cell culture (infection), RdRp *in vitro* assay	FCV
Pol (nucl.)	Favipiravir (T-705)	Cell culture (infection)	MNV
Pol (nucl.)	2-thiouridine	Co-crystallization w/pol	NV
Pol (nucl.)	5-nitrocytidine	Co-crystallization w/pol	NV
Pol (nucl.)	2′-amino-2′-deoxycytidine	Co-crystallization w/pol	NV
Pol (NNI)	Suramin	RdRp *in vitro* assay, co-crystallization w/pol, *in silico* docking	NV, MNV
Pol (NNI)	NF203	RdRp *in vitro* assay, co-crystallization w/pol, *in silico* docking	NV, MNV
Pol (NNI)	PPNDS	RdRp *in vitro* assay, co-crystallization w/pol	NV
Pro	Acyclic sulfamide-based compounds	*In vitro* protease assay, cell culture (replicon)	NV
Pro	Piperazine derivatives	*In vitro* protease assay, cell culture (replicon)	NV
Pro	Pyranobenopyrone compounds	*In vitro* protease assay, cell culture (replicon)	NV
Pro	Cyclosulfamide-based derivatives	*In vitro* protease assay, cell culture (replicon)	NV
Pro	Dipeptidyl or tripeptidyl transition state inhibitors	*In vitro* protease assay, cell culture (replicon), co-crystallization w/pro	NV, MNV
Pro	Chymostatin	*in vitro* protease assay	NV
Entry (HBGA)	Multiple compound classes	*in vitro* binding assay	NV
Entry (virus)	Monoclonal antibodies	*in vitro* binding assay, chimpanzee infection	NV
	Human interferon alpha	cell culture (replicon) genobiotic pig infection	NV
	Nitazoxanide (tizoxanide, Alinia®)	Phase II trial transplant patient	NV
	(E)-2-styrylchromones	Cell culture (infection)	MNV
Unfolded protein response	Deubiquitinase inhibitors	Cell culture (infection), cell culture (replicon)	MNV NV

Oral and intravenous immunoglobulins have been tried in immunosuppressed patients with variable success. While there have been several case series that demonstrated improvement in diarrheal symptoms within 2–3 days of administration of oral immunoglobulins, a more recent cohort study failed to demonstrate improvement in time to resolution of diarrhea, length of hospital stay, or cost of hospitalization with the administration of oral immunoglobulins.[55-57] Likewise, breast milk has had variable results and is currently undergoing study as a potential treatment of Norovirus (ClinicalTrials.gov Identifier: NCT02371538). Systemic administration of immunoglobulin has provided conflicting evidence of clinical impact on Norovirus infection.[1, 3] A novel monoclonal Norwalk-virus specific antibody has been developed and found to be effective in a chimpanzee model of infection, but efficacy in humans remains unproven.[58] A number of drugs that are currently approved, including nitazoxanide, ribavirin, suramin and human interferon-α have activity against Norovirus (Table 2). Several drugs including favipiravir in early phase of development show promise in the treatment of Norovirus (Table 2).[1, 3, 36]

Nitazoxanide (2-acetyloxy-*N*-(5-nitro-2-thiazolyl) benzamide Alinia®) is a thiazolide compound that is rapidly hydrolyzed to its active metabolite, tizoxanide, which has broad activity against bacteria, parasites and viruses.[59] Nitazoxanide is approved by the United States Food and Drug Administration as an oral suspension (age ≥ 1 year) and a 500 mg tablet (age ≥ 12 years) for treating diarrhea caused by *Giardia lamblia* and *Cryptosporidium parvum.* The nitazoxanide table contains 500 mg of nitazoxanide and the following inactive ingredients: maize starch, pregelatinized corn starch, hydroxypropyl methylcellulose, sucrose, sodium starch glycolate, talc, magnesium stearate, soy lecithin, polyvinyl alcohol, xanthan gum, titanium dioxide, FD&C Yellow No. 10 Aluminum Lake, FD&C Yellow No. 6 Aluminum Lake, and FD&C Blue No. 2 Aluminum Lake.

Pharmacology of Nitazoxanide

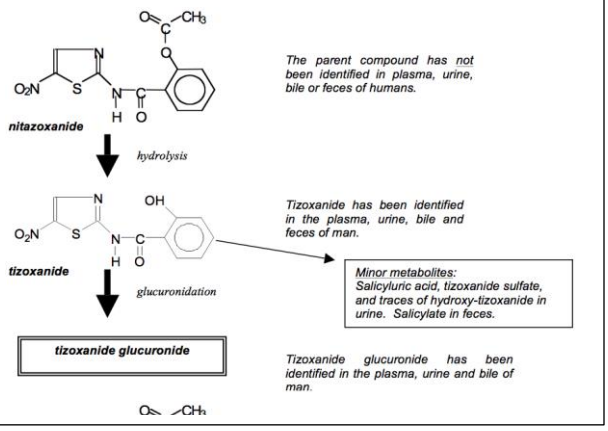
After oral administration, maximum plasma concentrations of the active metabolites tizoxanide and tizoxanide glucuronide are observed within 1-4 hours. The active metabolite elimination half-life is 7.3 hours. The parent nitazoxanide is not detected in plasma. Key pharmacokinetic parameters are summarized in Table 3. From this data, it is clear that the oral suspension is not bioequivalent to the tablet formulation. The relative bioavailability of the suspension compared to the tablet is 70%.

Table 3: Plasma Pharmacokinetic Values Following a Single Oral Dose of Nitazoxanide
[1]Mean (± SD). [2]Mean (Range)

Formulation	Dose	Age (Yrs)	Tizoxanide			Tizoxanide Glucuronide		
			C_{max}[1] (µg/mL)	T_{max}[2] (Hr)	AUC[1] (µg•hr/mL)	C_{max}[1] (µg/mL)	T_{max}[2] (Hr)	AUC[1] (µg•hr/mL)
Tablet	500mg	12-17	9.1 (6.1)	4.0 (1-4)	39.5 (24.2)	7.3 (1.9)	4.0 (2-8)	46.5 (18.2)
	500mg	≥18	10.6 (2.0)	3.0 (2-4)	41.9 (6.0)	10.5 (1.4)	4.5 (4-6)	63.0 (12.3)

[1]Mean (± SD). [2]Mean (Range)

Figure 3: Nitazoxanide Metabolism



Administration of the tablet with food increased the AUC_t of tizoxanide and tizoxanide glucuronide in plasma almost two-fold and the C_{max} by almost 50%. Likewise, administration of the suspension with food increases the AUC_t of tizoxanide and tizoxanide glucuronide by about 45-50% and the C_{max} by ≤10%. It is recommended to always take nitazoxanide with food to ensure maximal delivery of the active drug. Repeat dosing of nitazoxanide every 12 hours for 7 consecutive days failed to demonstrate significant accumulation of nitazoxanide metabolites tizoxanide or tizoxanide glucuronide detected in plasma.

In plasma, the parent drug, nitazoxanide is generally not detected while 99% of tizoxanide is bound to proteins. Following oral administration in humans, nitazoxanide is rapidly hydrolyzed to an active metabolite, tizoxanide (desacetyl-nitazoxanide). Tizoxanide then undergoes conjugation, primarily by glucuronidation. *In vitro* metabolism studies have demonstrated that tizoxanide has no significant inhibitory effect on cytochrome P450 enzymes. (Alinia® (nitazoxanide) Prescribing Information. Romark, L.C., Tampa, FL. November 2013)

Tizoxanide is excreted in the urine, bile and feces, and tizoxanide glucuronide is excreted in urine and bile. Approximately two-thirds of the oral dose of nitazoxanide is excreted in the feces and one- third in the urine. The pharmacokinetics of nitazoxanide in pediatric patients less than one year of age and geriatric patients has not been studied. Data in patients with impaired hepatic and renal function is limited. [60] The PK of nitazoxanide has been studied in patients given a single dose of nitazoxanide 600mg with mild renal impairment [eGFR 60-89 mL/min/1.73 m2], moderate renal impairment [eGFR 30-59 mL/min/1.73 m2], severe renal impairment [eGFR 15-29 mL/min/1.73 m2] and end stage renal disease requiring dialysis (6 subjects in each group). Terminal half-life and time to peak concentration for tizoxanide, the active metabolite, were similar between renal impaired patients and healthy subjects. There was a trend to increasing. C_{max} and AUCs for tizoxanide glucuronide, a non-active metabolite, increased with the degree of renal impairment. There were no treatment emergent adverse events that were related to the study drug (Nitazoxanide Investigator's Brochure, March 1, 2018). Similarly, there was a small phase I study of single dose nitazoxanide 600mg in patients with normal, mild (Child-Pugh Class A), moderate (Child-Pugh Class B) and severe hepatic

impairment (Child-Pugh Class C) (6 subjects in each arm). No clear relationships between tizoxanide and tizoxanide glucuronide PK parameters and hepatic function parameters (Child-Pugh classification and the individual components serum albumin, serum bilirubin and INR) were apparent. There were no treatment emergent adverse events (Nitazoxanide Investigator's Brochure, March 1, 2018).

Nitazoxanide has been extensively studied in multiple animal models to assess safety, pharmacokinetics and efficacy, as summarized in the attached IB. To date, 4,751 patients have been exposed to nitazoxanide in clinical trials, and the product has been marketed for approximately nine years in the United States and sixteen years in Latin America with more than 70 million people treated. While nitazoxanide is specifically approved for twice daily dosing for 3 days duration, there is extensive experience with more prolonged dosing, including dosing beyond 30 days (Alinia® (nitazoxanide) Prescribing Information. Romark, L.C., Tampa, FL. November 2013).

Adverse reactions to nitazoxanide are related primarily to the gastrointestinal tract with mild and transient abdominal pain (6.6-7.8%), diarrhea (2.1-4.2%), headache (1.1-3.1%), nausea (3.0%) and vomiting (1.1%) being the most common adverse events reported. Mild and transient yellow discoloration of the urine and sclera have also been reported, particularly in patients receiving doses higher than 500 mg bid for extended durations. Other rare adverse events occurring in less than 1% of patients include: asthenia, fever, pain, allergic reaction, pelvic pain, back pain, chills, infection, malaise, flu syndrome, dizziness, somnolence, insomnia, tremor, hyperesthesia, dyspepsia, anorexia, flatulence, constipation, appetite increase, enlarged salivary glands, dry mouth, thirst, dysuria, amenorrhea, metrorrhagia, kidney pain, edema labia, increased creatinine, increased alanine aminotransferase (ALT), anemia, leukocytosis, rash, pruritus, sweating, ear ache, epistaxis, lung disease, pharyngitis, rhinitis, tachycardia, syncope, hypertension, myalgia, leg cramps, and spontaneous bone fracture. No drug-related serious adverse events have been reported during post-marketing experience with nitazoxanide.[61-70]

While nitazoxanide has not shown signs of reproductive toxicity in animal studies, no adequate and well-controlled clinical studies have been conducted in pregnant or lactating females. It is not known whether nitazoxanide is excreted in breast milk. The drug is classified as pregnancy category B by the US FDA. No cases of overdose of nitazoxanide have been reported. Single doses of up to 4000 mg have been reasonably well tolerated in healthy volunteers. There is no specific antidote for nitazoxanide overdose, therefore, management of the patient should consist of symptomatic and supportive therapy.

Antiviral Activity of Nitazoxanide

Nitazoxanide and its circulating metabolite, tizoxanide, suppress the replication of a broad range of DNA and RNA viruses in cell culture including Norovirus. For viruses, the antiviral mechanism of nitazoxanide is believed to be due to targeting host-regulated processes involved in viral replication which leads to an inhibition of viral protein synthesis.[71-75] The relative activity of nitazoxanide and tizoxanide against enteric viruses is detailed in Table 4. Of great interest, the IC_{50} and IC_{90} for tizoxanide in a Norovirus G1 replicon-assay system using an HG-23 cell line were 0.5 and 1.2 µg/mL respectively.[75] While the precise mechanism of action of nitazoxanide has not been fully defined for Norovirus, tizoxanide appears to inhibit the maturation of rotavirus viral protein 7 (VP7), a glycoprotein that forming the outer part of the virion and one of the six structural glycoproteins involved in rotavirus replication, in addition to altering viroplasm formation and interfering with viral morphogenesis.[74] Nitazoxanide has also been shown to potentiate the production of type I interferons. The significance of this activity is not fully understood, but it could contribute to the antiviral activity of nitazoxanide by interfering with maturation of viral proteins or other mechanisms.[75]

Table 4: Activity of Nitazoxanide and Tizoxanide against Enteric Viruses in Cell Culture

Virus	TIZ EC_{50}	TIZ CC_{50}
Simian rotavirus SA11 - G3P (72, 74)	0.5 µg/ml	>50
Human rotavirus Wa - G1P (74)	1.0 µg/ml	>50
Norovirus G1 (75)	0.5 µg/ml	14

EC_{50} = drug concentration at which a 2-fold depression of viral DNA or RNA (relative to the average levels in untreated cultures) was observed. CC_{50} = drug concentration at with a 2-fold depression of reduced MTT was observed relative to average levels in untreated cultures.

Clinical Experience with Nitazoxanide in Treating Enteric Viruses

The efficacy and safety of nitazoxanide suspension as a treatment for diarrhea caused by enteric viruses was first studied in Egypt.[72] Children hospitalized with severe rotavirus gastroenteritis were randomized to either 7·5 mg/kg nitazoxanide as an oral suspension or placebo twice a day for 3 days. Patients were monitored in the hospital for 7 days. Of the 47 patients included in the efficacy analysis, 38 patients had rotavirus, 4 patients had adenovirus, 4 patients had rotavirus and adenovirus, and 1 patient had rotavirus and Norovirus at baseline. The median time to resolution of illness was 31 h (IQR 22–73) for the nitazoxanide-treated group compared with 75 h (51–124) for the placebo group (p=0·0137). Two adverse events were reported in patients randomized to the placebo group, one was a case of bronchitis and the other was a case of mild otitis media.

A second study evaluated the effect of nitazoxanide tablets for the treatment of viral gastroenteritis caused by Norovirus, rotavirus, or adenovirus in patients over the age of 12.[76] Of the 45 evaluable patients, 26 had rotavirus, 13 had Norovirus, 5 had adenovirus and 1 had both rotavirus and adenovirus detected. Patients were randomly assigned to receive either nitazoxanide 500 mg twice daily or placebo twice daily for 3 days. The results indicated the median time from first dose to resolution of symptoms was 1.5 days in the nitazoxanide treated patients and 2.5 days for the placebo group (P=0.0001). Time to symptom resolution was faster in nitazoxanide treated subjects who were infected with rotavirus (1.5 vs. 2.5 days, P=0.0052) and Norovirus (1.5 vs. 2.5 days, P=0.0295). The medication was well tolerated with no serious adverse events reported during this study.

Given the severity of Norovirus in immunosuppressed patients, there has been significant interest in using nitazoxanide to improve outcomes. One of the first published cases involved a 43-year-old male with relapsed refractory acute myelogenous leukemia who had undergone hematopoietic stem cell transplantation and had biopsy-proven cutaneous and pulmonary graft-versus-host disease. Shortly after initiating ponatinib for management of his AML, he presented with Norovirus infection associated with 8 – 12 daily episodes of large volume, non-dysenteric watery diarrhea but without fever, chills, or any associated abdominal pain or tenesmus. He was significantly lymphopenic on admission. He was also clinically unstable with tachycardia (115 bpm) and hypotensive (88/55 mm Hg). Stool studies were negative for all tested pathogens other than Norovirus. He failed to respond to antimotility treatment and octreotide but, within 24 hours of starting nitazoxanide 500mg twice daily, he had marked clinical improvement from 10 to 2 bowel movements per day. He had normal consistency and frequency of his bowel movements within 4 days of starting nitazoxanide. Clinical resolution of acute gastroenteritis was achieved with a 7 day course of nitazoxanide but the patient continued to asymptomatically shed Norovirus in his stools for over 30 days.[77]

Recently, one of the largest experiences of nitazoxanide for the treatment of Norovirus in immunosuppressed patients was presented at the 2015 BMT Tandem Meeting.[78, 79] This retrospective study of 14 patients (3 receiving chemotherapy, 2 autologous HSCT and 9 allogeneic HSCT) with Norovirus documented the impact of nitazoxanide was 100 mg PO BID for ages 1 to 4 years, 200 mg PO BID for age 4 to 11 years, and 500 mg PO BID for greater than 11 years. 4 patients received 500 mg PO BID for 5-8 days (Mean duration 4.6 days, median duration 5 days. Incidence of adverse events are not yet published. The patients, who were 1-21 years old, included 3 who were pre-HSCT on chemo/immunotherapy and 8 patients who were 1 day to 34 months after HSCT. All of the HSCT patients were on immunosuppressive therapy and 5 had documented GVHD. Therapy resulted in improvements in diarrhea, nausea, and

abdominal pain within 2-4 days (median 2 days). Clearance of stool virus was variable. Two of 3 patients treated prior to HSCT became negative on stool study within 5-14 days of treatment (1 unknown duration). Among patients treated after HSCT 4 of 9 had persistent viral shedding, 2 received drug until death (1 adenovirus, 1 congestive heart failure) both were treated greater than 2 months, 3 with GVHD continue to shed virus after 6 months of treatment, and 4 have come off therapy and remain negative for Norovirus RNA. One HSCT patient with clinical resolution but persistent viral shedding stopped treatment, resulting in recurrence of clinical symptoms. This patient responded clinically to reinstitution of therapy within 2 days but continued to shed virus. UGI endoscopy/ colonoscopy were performed in 5 pts at the time of infection, all showed inflammation/edema but no GVHD was seen on histology.

Clinical Experience with Nitazoxanide Used for Greater than 3 days

There have been 3 studies (n=505) using nitazoxanide along or in combination with interferon for the treatment of hepatitis C for 3-10 months and one study of prolonged nitazoxanide for patients with AIDS and Cryptosporidiosis who were given 1-1528 days (median 62 days) of nitazoxanide.[89-92] In the Cryptosporidiosis study, Fifty-six percent of patients reported a total of 482 adverse events during the course of the study.[92] Most adverse events (72%) were mild and deemed unrelated to the nitazoxanide. None of the deaths or other serious adverse events (life-threatening or requiring hospitalization) was reported to be related to the use of nitazoxanide. Twenty-seven non-serious adverse events were considered possibly related to the use of the study drug. Twenty of these events were associated with the digestive tract (nausea, vomiting, diarrhea, abdominal pain and dyspepsia). Other events possibly related to treatment were allergic reaction (n = 2), rash (n = 1), yellowish discoloration of the sclera (n = 2), and discoloration of urine (n = 2). Each of these events was transient in nature resolving on treatment or upon the discontinuation of treatment. In the 3 HCV studies, were attributed to the peginterfron and/or the ribavirin without apparent enhancement of the incidence in those patients who received nitazoxanide.[89-91]

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2.2 Rationale

Norovirus is a rare and emerging viral disease of medical importance in the immunocompromised subject. *In vitro,* nitazoxanide has been shown to be active against Norovirus. Results from double-blind placebo-controlled studies demonstrate that nitazoxanide decreases the duration of illness in subjects with viral gastroenteritis. Small observational studies suggest that nitazoxanide results in prompt improvement in clinical signs of Norovirus-induced gastroenteritis. Further, nitazoxanide enhanced local production of interferon which may contribute to more rapid clearance of virus. Studies of nitazoxanide for the treatment of Norovirus has presented limited data on quantitative virology and its impact on viral load are less well defined. Further, there are limited data on the impact of nitazoxanide on host and viral changes that may facilitate control and clearance of Norovirus.

The present study is a phase 2 multi-center, prospective, randomized, double-blind study designed to assess the efficacy of nitazoxanide taken twice daily for 28 days compared to placebo for the treatment of Norovirus gastroenteritis in immunosuppressed transplant subjects. Given the safety of prolonged therapy with nitazoxanide [89-92], lack of interactions with common post-transplant medications, putative antiviral activity and prolonged duration of viral shedding we are assessing 28 days of therapy. The longitudinal monitoring phase will provide useful information on the course of host and viral responses in subjects with chronic Norovirus infection with and without treatment.

There are 2 arms of the study:

Nitazoxanide Arm**:** All subjects age ≥ 12 years: 500 mg (one tablet) nitazoxanide by mouth twice daily with food for 28 consecutive days.

Placebo Arm: All subjects age ≥ 12 years: Placebo (one tablet) by mouth twice daily with food for 28 consecutive days.

2.3 Potential Risks and Benefits

2.3.1 Potential Risks

There are a number of potential risks associated with this study. Risk is primarily derived from the study agent and the protocol-mandated procedures.

Risks Associated with Nitazoxanide: As outlined in the package insert for the nitazoxanide, the compound has been extensively studied and utilized clinically in a wide range of clinical settings including limited data in transplant recipients. The safety of nitazoxanide was evaluated in 2177

HIV-uninfected subjects 12 months of age and older who received nitazoxanide Tablets or nitazoxanide for Oral Suspension at the recommended dose for at least three days. In pooled controlled clinical trials involving 536 HIV-uninfected subjects treated with nitazoxanide Tablets or nitazoxanide for Oral Suspension, the most common adverse reactions were abdominal pain, headache, chromaturia and nausea (>2%). Safety data were analyzed separately for 280 HIV-uninfected subjects' ≥12 years of age receiving nitazoxanide at the recommended dose for at least three days in 5 placebo-controlled clinical trials and for 256 HIV-uninfected subjects 1 through 11 years of age in 7 controlled clinical trials. There were no differences between the adverse reactions reported for nitazoxanide -treated subjects based upon age.

The following adverse reactions have been identified during post approval use of nitazoxanide. Because these reactions are reported voluntarily from a population of uncertain size, it is not always possible to reliably estimate their frequency or establish a causal relationship to drug exposure. The following is a list of adverse reactions spontaneously reported with nitazoxanide Tablets which were not included in clinical trial listings:

Gastrointestinal disorders: diarrhea, gastroesophageal reflux disease.
Nervous System disorders: dizziness.
Respiratory, thoracic and mediastinal disorders: dyspnea.
Skin and subcutaneous tissue disorders: rash, urticaria.

Risks Associated with Protocol-Mandated Procedures:

Blood Samples:	Drawing blood from a vein may cause local pain, bruising, occasional light-headedness, fainting, and very rarely, infection at the site of the blood draw.
Buccal Swabs:	Buccal swabs may cause discomfort, irritation, or a gagging sensation.
Stool Collection:	Providing a stool specimen might make the subject feel embarrassed.
Urine Test:	Female who could become pregnant, providing urine for a pregnancy test might feel embarrassed.
Questionnaires:	Completion of a questionnaire and the questions might make the subject feel uncomfortable or upset.

Others:

Pregnancy: There are no data with this drug in pregnant women to inform a drug-associated risk. No teratogenicity or fetotoxicity was observed in animal reproduction studies with administration of nitazoxanide to pregnant rats and rabbits during organogenesis at exposures 30 and 2 times, respectively, the exposure at the maximum recommended human dose of 500 mg twice daily based on body surface area (BSA).

In the U.S. general population, the estimated background risk of major birth defects and miscarriage in clinically recognized pregnancies is 2% to 4% and 15% to 20%, respectively.

Breach of Confidentiality: Instances like lost or stolen laptops storing participant information, lost or stolen thumb drives, paperwork with subject PHI left unsecured may result in breach in confidentiality.

Unknown risks: Nitazoxanide and protocol mandated procedures may have risks previously unknown.

2.3.2 Known Potential Benefits

Given the limited clinical experience with nitazoxanide, there is no guarantee that subjects will receive direct benefit from participating in this study. It is possible that nitazoxanide will reduce the duration of symptoms secondary to Norovirus infection and may also reduce the duration of Norovirus shedding. There have been no prospective studies of Norovirus in the transplant setting and this study will allow a better assessment of the natural history of Norovirus in this setting. Lastly, collection of various immune response markers will allow assessment of the relative role of the host immune response and antiviral therapy to recovery from Norovirus infection in transplant recipients. All subjects will get the local standard of care provided to patients with Norovirus (i.e. antimotility agents and hydration).

3 OBJECTIVES

3.1 Study Objectives

A. Primary Objective:

1. To assess the *clinical efficacy* of nitazoxanide for the management of acute and chronic Norovirus in transplant recipients.

B. Secondary Objectives

1. To assess *virologic efficacy* of nitazoxanide
2. To assess the *safety* of nitazoxanide for the management of acute and chronic Norovirus in transplant recipients.

C. Exploratory Objectives

1. To assess *initial clinical improvement* in Norovirus Disease through 28 and 180 days.
2. To assess *markers of virologic improvement* Norovirus Disease through 28 and 180 days.
3. To assess the *contribution of immunologic response* to Norovirus to the clinical course of disease in treated and untreated subjects.
4. To define the *pharmacokinetics and dose response relationships* of nitazoxanide.
5. To define the *natural history* of Norovirus in nitazoxanide treated and untreated subjects.

3.2 Study Outcome Measures

No studies have been conducted to establish the optimal endpoint for assessing novel drugs for the treatment of Norovirus in immunosuppressed subjects. Further, there are no published FDA guidance documents to inform optimal endpoint selection for this indication.[1] Available literature and expert opinion was consulted to define proposed endpoints for this study.

3.2.1 Primary Outcome Measures

1. The time from randomization until initial clinical resolution of Norovirus symptoms for at least 48 hours through 180 days.

Clinical resolution will be assessed from the daily diary by the subject and will be defined as:

a. Cessation of vomiting and,
b. No stools classified by the Bristol Stool Chart as diarrhea (Type 6 or 7)

3.2.2 Secondary Outcome Measures

Virologic Efficacy

1. Time from randomization to first negative viral load through 180 days.
2. The change in viral titer between Day 1 and 180.

Safety

3. Incidence of unsolicited non-serious adverse events (see section 9 for details) through 60 days.
4. Incidence of laboratory adverse events (WBC, Hemoglobin, Platelet Count, Creatinine, Alkaline Phosphatase, ALT, AST, BUN and Bilirubin) through 60 days.
5. Incidence of protocol-specified serious adverse events (see section 9 for details) through 60 days.
6. Incidence of Hospitalization through 60 days.

3.2.3 Exploratory Outcome Measures

Clinical Improvement;

1. Time from randomization to initial clinical improvement in Norovirus disease through 28 and 180 days for at least 48 hours, as defined by:
    a. 50% reduction in number of episodes of vomiting and,
    b. Improvement in PO intake, as judged by the subjects and,
    c. 50% reduction in the number of episodes of diarrhea as classified by the subject by the Bristol Stool Chart as type 6 or 7 bowel movements.
2. Time from randomization to initial cessation of vomiting through Day 180 (recurrent vomiting will be considered disease recurrence unless determined to be due to another etiology [i.e. GVHD]).
3. Time from randomization to subject report of no loss of appetite that is maintained for 48 hours through Day 180 (measure will be first time that the subject reports that they have no “loss of appetite” on their daily diary).
4. Time from first dose to initial cessation of diarrhea through Day 180 classified by the Bristol Stool Chart as type 6 or 7 bowel movements (recurrent diarrhea will be considered disease recurrence unless determined to be due to another etiology [i.e. GVHD]).
5. Time from randomization to 50% reduction in the amount of antimotility agents utilized by the subject through Day 180
6. Number of days of diarrhea through Days 28 and 180.
7. Number of stools classified by the Bristol Stool Chart as type 6 or 7 bowel movements through Day 28.
8. Number of days of IV hydration or total parenteral nutrition (TPN) therapy through Day 28.

9. Total number of days of hospitalization through Days 28 and 180.
10. Change in patient-reported quality of life as measured by EuroQOL-5 (global) and IBSQOL (diarrhea specific) [adult] or EuroQOL-5 Peds (global) and PedsQL GI Module [children] from baseline to Day 180.
11. Change in patient-reported physical function as measured by NIH PROMIS Physical Function (Adult) Pediatric Global Health (Peds) from baseline to Day 180.
12. Change in patient-reported emotional distress as measured by the NIH PROMIS Depression, Anxiety, Fatigue (Adult and Peds) from baseline to Day 180.
13. Change in patient-reported gastrointestinal symptoms as measured by the NIH PROMIS GI (Adult and Peds) symptoms (6 of 8 subscales) from baseline to Day 180.
14. The effect of co-pathogens, as determined by the BioFire stool pathogen screen, on the primary outcome measure.
15. Time from randomization until initial clinical resolution of Norovirus symptoms for at least 48 hours through Day 180. Clinical resolution will be assessed from the daily diary by the subject and will be defined as cessation of vomiting and no stools classified by the Bristol Stool Chart as diarrhea (Type 6 or 7) from the total population compared to the subgroup of subjects who do not report chromaturia (discoloration of urine) based on unsolicited self-reporting.
16. Incidence of unsolicited non-serious adverse events (see section 9 for details) through 60 days, laboratory adverse events (WBC, Hemoglobin, Platelet count, Creatinine, Alkaline Phosphatase, ALT, AST, BUN and Bilirubin) through 60 days, protocol-specified serious adverse events (see section 9 for details) through 60 days, and Hospitalization through 60 days from the total population compared to the subgroup of subjects who do not report chromaturia (discoloration of urine) based on unsolicited self-reporting.

Virologic Improvement:

17. Time to ≥ 1 log reduction in stool Norovirus genome copies.
18. Change in quantitative Norovirus load in the stool between Day 1 and Day 7, 14, 21, 28, 60, 120, and 180.
19. Change in Norovirus sequence from Day 1 to Days 28 and 60.
20. The effect of co-pathogens, as determined by the BioFire stool pathogen screen, on the time from the randomization to first negative viral load through 180 days.
21. Time from the randomization to first negative viral load through 180 days and the change in viral titer between Day 1 and 180 from the total population compared to the subgroup of subjects who do not report chromaturia (discoloration of urine) based on unsolicited self-reporting.

Immunologic Response:

22. Change in Norovirus-specific serum and stool IgA, IgM and IgG between Day 1 and Day 28 and 60 (stool only).
23. Association between change in total lymphocyte count and T cell subsets at Day 1 and 180 and clinical resolution of Norovirus symptoms.
24. Association between change in total lymphocyte count and T cell subsets at Day 1 and 180 and undetectable quantitative norovirus PCR.
25. Association between change in Norovirus-specific serum and stool IgA, IgM and IgG from Day 1 to Days 28 and 60 (stool only) and clinical resolution of Norovirus symptoms.
26. Association between change in Norovirus-specific serum and stool IgA, IgM and IgG from Day 1 to Days 28 and 60 (stool only) and undetectable quantitative Norovirus PCR.

Pharmacokinetics and Dose Response:

27. For subjects consenting to PK draws, concentrations of nitazoxanide metabolites (tizoxanide and tizoxanide glucuronide) 1 to 4 hours after the first dose on Day 7 and 10 minutes before the second dose on Day 21.

Natural History of Disease:

28. Time to allograft rejection as per each center team
29. Time to allograft loss as reported to UNOS.
30. Time to death.
31. Time to withdrawal from the study because of intolerance or drug related adverse events.
32. Correlation of secretor status as defined by phenotype and genotype, separately, and clinical resolution of Norovirus as defined by the primary endpoint.

4 STUDY DESIGN

The study will be a phase 2 multi-center double-blind placebo-controlled study of the efficacy and safety of nitazoxanide for the treatment of solid organ and hematopoietic stem cell transplant recipients with symptomatic diarrhea due to Norovirus. The study will involve approximately 160 subjects randomly assigned (1:1) to nitazoxanide or placebo. The study duration is 60 months and subject participation duration is 6 months. Given the safety of prolonged therapy with nitazoxanide, lack of interactions with common post-transplant medications, putative antiviral activity and prolonged duration of viral shedding we are assessing 56 doses of therapy. The longitudinal monitoring phase will provide useful information on the course of host and viral responses in subjects with chronic Norovirus infection with and without treatment.

The primary endpoint
is to determine the *clinical efficacy* of nitazoxanide that will be assessed as the time from randomization until clinical resolution of Norovirus symptoms for at least 48 hours, assessed through 180 days. Secondary endpoints include *virologic efficacy* of nitazoxanide that will be assessed as the time from randomization to first negative viral load through 180 days and by comparing the change in viral titer between Day 1 and 180; *safety* of nitazoxanide that will assessed by the frequency of adverse events and serious adverse events.

There are 2 arms of the study:

Nitazoxanide Arm: All subjects age ≥ 12 years: 500 mg (one tablet) nitazoxanide by mouth twice daily with food for 56 consecutive doses.

Placebo Arm: All subjects age ≥ 12 years: Placebo (one tablet) by mouth twice daily with food for 56 consecutive doses.

5 STUDY ENROLLMENT AND WITHDRAWAL

Approximately 160 males and females ≥ 12 years of age inclusive, who meet all eligibility criteria will be enrolled. All candidates at all centers that are eligible to be included in the studies will be offered the opportunity to participate in the trial. Study subjects are seen in patient or clinic setting and have either community onset or hospital acquired disease.

5.1 Subject Inclusion Criteria

Subjects should meet all of the following inclusion criteria

1. Male or female age ≥ 12 years.
2. Recipient of a solid organ or hematopoietic stem cell transplant.
3. Positive test result for Norovirus within 14 days of enrollment that is obtained as part of routine clinical care using a Norovirus testing available to the site.
4. Active GI symptoms (diarrhea or vomiting) that, in the opinion of the PI, are secondary to Norovirus.[#]
5. Willing and able to provide written informed consent and assent before initiation of any study procedures, consistent with local IRB policy.
6. Subjects must be of non-childbearing potential or if of childbearing potential, must be using an effective method of birth control or must be abstinent.
   - Non-childbearing potential is defined as surgically sterile or postmenopausal for > one year.
   - Effective methods of birth control include the use of hormonal or barrier birth control such as implants, injectable contraceptives, combined oral contraceptives, intrauterine devices [IUDs] or condoms with spermicidal agents during study period. Female subjects must be using an effective method of birth control or practice abstinence and must agree to continue such precautions during the study and for 30 days after the Day 28 study visit.
   - A woman is eligible if she is monogamous with a vasectomized male. This subject is considered low risk and not required to use contraception.
7. Agrees to complete all screening requirements, study visits and procedures.

[#] Patients must have active diarrhea, which is defined as at least 3 days of Bristol 6 or 7 stools in the past 2 weeks prior to enrollment per patient report.

5.2 Subject Exclusion Criteria

Subjects meeting any of the exclusion criteria at baseline will be excluded from study participation

1. Other identified infectious causes of diarrhea at screening*.
2. Any condition that would, in opinion of the Site Investigator, place the subject at an unacceptable risk of injury or render the subject unable to meet the requirements of the protocol.
3. Subjects receiving oral or intravenous immunoglobulin therapy concurrently or in the 14 days prior to enrollment.
4. Nitazoxanide use for any illness in the previous 30 days prior to randomization.
5. Have received experimental products within 30 days prior to the study entry or plan to receive experimental products at any time during the study
6. Known sensitivity to nitazoxanide or any of the excipients comprising the nitazoxanide tablets.
7. Subjects unable to swallow oral medications.
8. Subjects with ostomy.
9. Women who are pregnant or lactating or have a positive urine pregnancy test at screening/enrollment/Day 1.

Co-enrollment in other studies is allowed if none of the above exclusion criteria are met.

*Alternative diagnosis requiring treatment would be considered a co-infection; if the testing is positive for a pathogen that the PI does not feel is causing the symptoms, they may be included but the PI or his/her designee must document that the positive test is not clinically significant, does not require treatment and is not causing the symptoms making the patient eligible for enrollment.

5.3 Treatment Assignment Procedures

5.3.1 Randomization Procedures

Enrollment of subjects will be done online using the enrollment module of AdvantageEDC^{SM}. Approximately 160 subjects will be randomized into two treatment groups: active nitazoxanide and placebo with equal allocation (1 to 1 randomization) after providing consent and confirmation of eligibility based on the study inclusion and exclusion criteria.

The study will use a stratified, permuted block randomization scheme. Permuted block randomization is used to avoid the potential for serious imbalance in the number of participants assigned to each group, an imbalance that can occur in the simple randomization procedures.

Stratification will be by:

- Duration of Symptoms: onset of Norovirus-associated symptoms (diarrhea, nausea and/or abnormal appetite) < 14 days vs. ≥ 14 days
- Transplant Type: solid organ vs. hematopoietic stem cell transplant
- Age Range: pediatric (12-17 years) vs. adult ≥ 18 years

The randomization list will be computer-generated by statisticians at the SDCC using a stratified, permuted block randomization scheme and is included in the enrollment module for the trial. AdvantageEDC^{SM} will assign each subject to a treatment group after the demographic, duration of symptoms, transplant type, and eligibility data have been entered into the system.

Each site will have a supply of two 60cc identical blinded bottles pre-labeled with randomization numbers, each containing 30 tablets, sufficient to treat a subject with 56 doses. The two identical containers will be packaged in the same plastic bag. Once a subject is assigned a randomization number, the corresponding bottles will be distributed to the subject.

5.3.2 Masking Procedures

All subjects will take either nitazoxanide or matching placebo twice daily for 56 doses. The placebo will be manufactured by Romark, L.C., Ltd. to match the appearance (color, size, with "CTM" imprints) of the active compound and will be dispensed in identical bottles. Bottles will be labeled in such a way that it will not be obvious to the care team or the subject which arm they have been randomized to. Bottles will be delivered to the site pre-filled containing either nitazoxanide or placebo and will have a unique number. At the time of randomization, the unique number will be assigned to the individual subject. No member of the study team will be aware of the arm that the subject has been assigned to unless the site PI specifically requests unblinding (see section 5.3.4).

Refer to the MOP for unblinding procedures, including emergency unblinding procedures (Section 5.2 of MOP).

5.3.3 Reasons for Withdrawal and Discontinuation of Study Product Administration

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:

- Subject no longer meets eligibility criteria
- Subject withdraws consent
- Subject not able to comply with study procedures
- Subject becomes noncompliant
- Evidence of toxicity including study drug-related AE/SAE, laboratory abnormality, intercurrent illness, or other medical condition or situation occurs that continued participation in the study would not be in the best interest of the subject.
- 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
- Subject lost to follow-up
- Decision by the Investigator that termination is in the subject’s best medical interest.
- Decision by the sponsor to stop or cancel the study
- Decision by local regulatory authorities (IRB/IEC) to stop or cancel the study
- 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)

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.

5.3.4 Handling of Withdrawals

Subjects who withdraw or are withdrawn from participation in the study at any time. Subjects may also withdraw voluntarily from receiving the study intervention for any reason. An investigator may also withdraw a subject from receiving further intervention

Subjects who withdraw, or are withdrawn from the efficacy endpoint analysis, or are lost to follow-up after signing the informed consent form, randomization, and receipt of study product will not be replaced. Subjects who withdraw consent after signing the informed consent form and randomization but before receipt of study product may be replaced.

If a subject withdraws from the study after receiving any amount of study drug the subject will be asked to continue scheduled study procedures including safety and efficacy evaluations, if possible, and be given appropriate care under medical supervision if symptoms of any AE related to participation in the study are continuing. The participant will be followed until the AE is resolved or until the subject's condition becomes stable.

Subjects who request to withdraw their consent after study treatment from further participation in the study will be reminded of the importance of continuing the study for safety evaluations and will be encouraged to complete the Early Termination Visit if they choose not to complete the remaining study visits. The Early Termination Visit procedures are listed in Section 7.4. Subjects who choose to decline continuation in study participation will no longer be contacted for follow-up.

If a subject is withdrawn at any time due to an AE, the event will be followed until it is resolved or in the opinion of the Investigator is determined medically stable with the consent from the subject. The site PI may request unblinding of the study drug assignment if they feel that such information is essential for the management of an AE or SAE. If the PI feels that unblinding is essential for management of the AE or SAE, they will request unblinding by contacting the DMID Medical Monitor. Clinical failure is not an acceptable reason to request unblinding.

Refer to the MOP and the DMID policy "Unblinding Individual Participants in DMID Clinical Research," the current policy is available at https://www.niaid.nih.gov/research/dmid-data-management.

In the case of subjects who fail to appear for a follow-up safety assessment, extensive effort (i.e., three documented contact attempts via phone calls, e-mails, etc., made on separate occasions and followed by a certified letter) will be made to locate or recall them, or at least to determine their health status. Although the subject is not obliged to give reasons for withdrawing early, the

investigator should make a reasonable effort to ascertain the reasons while fully respecting the subject's rights. These efforts will be documented in the subject's records.

5.3.5 Termination of Study

The study will be terminated if the DSMB determines that the study should be terminated based on safety concerns. If the study is terminated, the DSMB will be permitted to allow the longitudinal monitoring phase of the study to proceed. DMID and/or the IND holder may terminate the study at their sole discretion. Although the sponsor had every intention of completing this trial, it reserves the right to terminate this trial at any time for clinical or administrative reasons. Reasons for termination include, but not limited to, study closure due to DSMB review and recommendation and at the discretion of DMID.

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.

5.3.6 Recruitment and Retention

Study subjects are seen either inpatient or clinic setting, and the Transplant Infectious Disease team is notified and consulted on all patients diagnosed with Norovirus. All candidates at all centers that are eligible to be included in the study will be offered the opportunity to participate in the trial.

The transplant patient population have a proven track record of a high compliance and retention rate in research related activities. The study subject clinical appointments will be adjusted to coincide with their research visit windows to ensure convenience. In addition, the subjects will be compensated for their research study visit.

6 STUDY INTERVENTION/INVESTIGATIONAL PRODUCT

6.1 Study Product Description

Nitazoxanide is a synthetic antiprotozoal agent for oral administration. It is designated chemically as 2-acetyloxy-N- (5-nitro-2-thiazolyl) benzamide. The molecular formula is $C_{12}H_9N_3O_5S$ and the molecular weight is 307.3. The placebo will look identical to nitazoxanide, and will have the same inactive ingredients (described in section 6.1.2).

6.1.1 Acquisition

Romark, L.C. will provide any necessary nitazoxanide 500mg tablets and matching placebo at no cost. Upon request from DMID, the study products (nitazoxanide and placebo) will be shipped to the following address:

DMID Clinical Materials Services (CMS)
Fisher BioServices
20439 Seneca Meadows Parkway
Germantown, MD 20876
Phone: 240-477-1350
Fax: 240-477-1360
Email: DMID.CMS@ThermoFisher.com

Study products, nitazoxanide 500mg tablets and matching placebo will be shipped from DMID CMS to investigational site upon request and approval by DMID.

6.1.2 Formulation, Packaging, and Labeling

Nitazoxanide Tablets

Nitazoxanide will be supplied as a 500 mg round, yellow, film-coated tablet with "CTM" imprint, manufactured by Romark, L.C. Per the package insert, inactive ingredients include: maize starch, pregelatinized corn starch, hydroxypropyl methylcellulose, sucrose, sodium starch glycollate, talc, magnesium stearate, soy lecithin, polyvinyl alcohol, xanthan gum, titanium dioxide, FD&C Yellow No. 10 Aluminum Lake, FD&C Yellow No. 6 Aluminum Lake, and FD&C Blue No. 2 Aluminum Lake.

Placebo Tablets

Placebo will be supplied as a matching tablet of the active drug which will also be a round, yellow, film-coated tablet with "CTM" imprint. Ingredients include: lactose monohydrate, magnesium stearate, talc, soy lecithin, polyvinyl alcohol, xanthan gum, titanium dioxide, FD&C

Yellow No. 10 Aluminum Lake, FD&C Yellow No. 6 Aluminum Lake, and FD&C Blue No. 2 Aluminum Lake. In order to maintain the blind, the placebo will be formulated for the same appearance as the active study drug.

Package and Labeling

All study product will be packaged in two 60cc identical containers each containing 30 tablets. Each container will also be labeled in compliance with applicable regulatory requirements, including the FDA-required cautionary statement *"Caution: - New drug -Limited by Federal Law to Investigational Use."*

6.1.3 Product Storage and Stability

Nitazoxanide and Placebo for Nitazoxanide tablets must be stored at 25°C (77°F); excursions are permitted to 15-30°C (59-86°F). [See USP Controlled Room Temperature].

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 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 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 productare 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.

For subjects in the hospital, the participating sites will maintain a temperature log to record temperature once per day in the room where the study products are stored for the period that the patient is in the hospital.

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

Nitazoxanide (administered orally as one 500 mg tablet) or placebo (administered orally as a matching tablet) twice daily with food for 56 doses. If the patient is NPO, product can be taken with water.

Subjects in the hospital will be provided the study drug consistent with the site SOP while ambulatory subjects will be provided two bottles, each containing 30 tablets.

A Site Research Pharmacist may be delegated the responsibility of study product dispensation. The Research Pharmacist must be a licensed, registered pharmacist and is the preferred healthcare practitioner to be delegated to perform this activity. If a Research Pharmacist is not available, a physician, nurse practitioner, physician assistant, registered nurse, or other authorized healthcare practitioner who is a member of the clinical study staff may be delegated to dispense the study product. These personnel must be licensed, trained, and qualified to prepare investigational study product and must be authorized to dispense the study product under state and local rules and regulations.

6.3 Modification of Study Intervention/Investigational Product for a Participant

There will be no modification of the dose of nitazoxanide during the 56 dose treatment phase. Subject's discontinuation of study treatment will be determined by the study site physician.

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

Study product will be stored and shipped from the DMID contract repository, Fisher BioServices, to the Clinical Sites. Once received, study product will be stored in and dispensed by the Investigational Pharmacy.

Used/opened product will be counted and disposed in accordance with site's SOPs with two staff members (two research pharmacists or a research pharmacist and/or a study team member) present co-signing and verifying the count (see MOP for additional detail). In addition, this verification form will be added to the protocol file so that DMID monitors can verify when at the sites for a routine IMV. In addition, used/opened product can also be retained until monitored and released for disposition, as applicable.

The United States 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 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.

Unused investigational product will be stored at 25°C (77°F); excursions permitted to 15-30°C (59-86°F) [See USP Controlled Room Temperature] in the Investigational Pharmacy until monitored and released for disposition as applicable. At study completion or termination, all unused investigational product will be disposed in accordance with the MOP following complete drug accountability and monitoring. Final disposition of the unused study product will be determined by DMID and communicated to the participating sites by the DMID Clinical Project Manager.

6.5 Assessment of Subject Compliance with Study Intervention/Investigational Product

Subjects will be requested to bring study drug with them to all visits during the Treatment Phase. Remaining pill count will be done by the site staff during each visit to assess compliance. If subjects forget to bring in their study drug or they lose their container, subject interview and study diary will be used to confirm medication adherence.

6.6 Concomitant Medications/Treatments

Subjects may receive any therapy that is deemed appropriate to treat the signs and symptoms of Norovirus after randomization at the discretion of their treating provider except for nitazoxanide and oral immunoglobulin. This can include:

- Intravenous immunoglobulin
- Changes in immunosuppression
- Anti-motility agents

Any treatment utilized should be included on the concomitant medications at the time of enrollment. Any concomitant medication pre- or post-randomization that is being used to treat the signs or symptoms of Norovirus should be marked as such on the Case Report Form for the entire duration of the study. Likewise, any change to immunosuppressive therapy would have a

note in the Case Report Form noting if the change was made because of elevated drug levels, for treatment of Norovirus or for other purposes. Concomitant medications will be collected through visit 12 (Day 180 after initial dose of study treatment) or early termination, whichever occurs first. If the Investigator learns that the subject has taken a prohibited medication (see inclusion/exclusion criteria) prior to the last dose, the Investigator will contact the DMID Medical Officer and CPM for instructions regarding the subject's continuation in the study.

Medications that might interfere with the evaluation of the investigational product should not be used unless absolutely necessary.

7 STUDY SCHEDULE

The study will need to enroll 160 subjects who meet inclusion and exclusion criteria and agree to participate in the study (*Section 5.1, 5.2-Subject Inclusion Criteria, Subject Exclusion Criteria*).

Upon enrollment in the study, subjects will be randomized into two treatment groups: active nitazoxanide and placebo:

a. Nitazoxanide Arm: All subjects age ≥ 12 years: 500 mg (one tablet) nitazoxanide by mouth twice daily with food for 56 consecutive doses.
b. Placebo Arm: All subjects age ≥ 12 years: Placebo (one tablet) by mouth twice daily with food for 56 consecutive doses.

Randomization will be stratified by age group (adult vs. pediatric), chronicity of Norovirus-associated symptoms (acute vs. chronic) and transplant type (HSCT vs. SOT) as follows:

- Duration of Symptoms: onset of Norovirus-associated symptoms (diarrhea, nausea and/or abnormal appetite) < 14 days vs. ≥ 14 days
- Transplant Type: solid organ vs. hematopoietic stem cell transplant
- Age Range: pediatric (12-17 years) vs. adult ≥ 18 years

Both investigators and subjects will be blinded to study treatments. Subjects will take study product preferably with food for 56 consecutive doses. If the study subject has an episode of emesis or spits out the study medication within one hour of administration, the event will be documented in the subject's record and then the full dose will be re-administered one time only per scheduled dose. All subjects must have therapy discontinued after taking 56 doses.

If a subject misses a dose, they will be instructed to skip that dose and resume taking their next dose as scheduled and to record the missed dose on their Subject Diary.

If symptoms recur after discontinuing therapy, subjects can resume any therapy at the discretion of the principal investigator or his/her designee with the exception of nitazoxanide. Nitazoxanide can be used after Day 28 if the local PI attests that it is medically urgent to use the nitazoxanide because the patient is experiencing severe or life-threatening disease. Although nitazoxanide can be used if it is felt critical to the subjects care, its use should be avoided until Day 180 whenever felt to be safe to do so by the local PI. Any use of nitazoxanide beyond Day 28 will be documented on the concomitant medication form and prescribed from commercial

stock and not provided by the study. Additional stool specimens should be collected prior to initiation of therapy for testing in the central lab when feasible.

The study will have 2 phases:

1. Treatment Phase (Study Day 1-28)
2. Longitudinal Monitoring Phase (Study Day 29-180)

7.1 Screening/Baseline/Treatment Phase

Any subject ≥ 12 years of age, recipient of a solid organ or hematopoietic stem cell transplant with a positive test for Norovirus within 7 days as part of routine clinical care will be approached to participate in the study. Once consent is obtained, then laboratory testing and medical history will be reviewed to determine eligibility unless local IRB waiver allows to review data prior to consent. Assessment required for eligibility may not be measured more than twice.

a. Screening/Enrollment/Baseline

- Consent/Assent will be reconfirmed with eligible subjects, consistent with local IRB policy for consent and assent of subjects.
- Subject and their chart will be reviewed to collect a medical history, concurrent medications.
- Urine pregnancy test will be obtained from women of childbearing potential. Results must be known and negative prior to dosing.
- WBC, TLC (Total Lymphocyte Count) Hb, Platelets (Derived from Complete Blood Count -within 3 days of enrollment).
- Creatinine, Alk Phos, AST, ALT, BUN, Bilirubin (Derived from Complete Metabolic Panel-within 3 days of enrollment).
- T Cell Subsets will be drawn unless CBC shows an ALC <100.
- Samples to be sent to the central laboratory are collected and processed:
    - Buccal swab and saliva samples for secretor assay.
    - Stool for quantitative Norovirus RT-PCR and sequencing.
    - Stool for other pathogen testing.
    - Stool for Norovirus-specific IgA, IgG, and IgM studies.
- Serum for quantitative Norovirus-specific IgA, IgG, and IgM and total IgG.
- Randomization.
- Vital signs will be obtained (pulse, temperature, BP, respiration, height and weight).
- Weekly Recall Packet will be administered.
- Detailed Subject Reported Outcome and Severity Score Assessment.
- Physical examination will be performed.

- Plastic bag with two 30-count bottles of study product will be dispensed to the subject. The first dose of study product (nitazoxanide or placebo) will be given by the study team.

The study product (nitazoxanide or placebo) will be taken by the subject twice daily for 28 days.

Subjects will be given a paper diary (to inquire about symptoms and severity along with overall well-being) that will be completed daily, at the time of study product dosing. The time of study product dosing will also be recorded. This will be completed on a daily basis through day 28. The pack of paper diary along with instructions that is given at enrollment will cover the entire study period.

The stool sample must have been produced within 24 hours of the visit and if it exceeds 24 hours it must be disposed. Subject would be encouraged to provide another sample within the visit window and if not, a rectal swab may be used for virus detection.

b. Study Day 7 ± 3 days

- Collection and review of completed diary pages.
- WBC, TLC, Hb, Platelets (Derived from Complete Blood Count).
- Creatinine, Alk Phos, AST, ALT, BUN, Bilirubin (Derived from Complete Metabolic Panel).
- Pill count to assess adherence.
- Samples of stool (collected at home or at clinic) to be sent to the central laboratory are collected and processed:
    - Stool for quantitative Norovirus RT-PCR and sequencing.
    - Stool for other pathogen testing; stool will be stored for later testing and this assay will not be performed for formed stool after Day 1.
- Nitazoxanide PK drawn 1-4 hours after the subject takes his/her dose, for subjects consenting to this optional assessment. The time of the blood draw and the time of the previous dose will be recorded.
- Vital signs (temperature, BP, respiration, weight and pulse) will be obtained.
- Weekly Recall Packet will be administered (Table 5).
- Collect concurrent medications.
- Inquiry about hospitalization since last assessment.
- Assess for adverse events.

c. Study Day 14 ± 3 days: Virtual Visit

- Team to call subject

- Review of completed diary pages.
- Subject to collect stool sample and store in freezer until day 28 study visit
- Weekly Recall Packet will be administered (Table 5).
- Collect concurrent medications.
- Inquiry about hospitalization since last assessment.
- Assess for adverse events.

d. Study Day 21 ± 3 days- Virtual Visit except for patients who agree to optional PK study

- Team to call subject not enrolled in the PK Sub Study (those patients will have in person visit)
- Review of completed diary pages.
- Subject to collect stool sample and store in freezer until day 28 study visit.
- For patients in the PK Sub Study:
  - Samples of stool to be sent to the central laboratory are collected and processed:
    - Stool for quantitative Norovirus RT-PCR and sequencing.
    - Stool for other pathogen testing; stool will stored for later testing and this assay will not be performed for formed stool after Day 1.
  - Nitazoxanide PK drawn 10 minutes before the subject takes his/her dose, for subjects consenting to this optional assessment. The time of the blood draw and the time of the previous dose will be recorded.
- Weekly Recall Packet will be administered (Table 5)
- Collect concurrent medications.
- Assess for adverse events.
- Inquiry about hospitalization since last assessment.

e. Study Day 28 + 3 days

All subjects should have dosing of medication stopped after 56 doses and residual medication returned. If this occurs after the day 28 visit, the patient should be informed of the date and time of the last dose and return the residual medications and bottles on Study Day 60.

- Collection and review of completed diary pages.
- WBC, TLC, Hb, Platelets (Derived from Complete Blood Count).
- Creatinine, Alk Phos, AST, ALT, BUN, Bilirubin (Derived from Complete Metabolic Panel).
- Samples of stool (collected at home or at clinic) to be sent to the central laboratory are collected and processed:
  - Stool for quantitative Norovirus RT-PCR and sequencing.

    - Stool for other pathogen testing; stool will stored for later testing and this assay will not be performed for formed stool after Day 1.
    - Stool Norovirus-specific IgA, IgG, and IgM studies.
- Serum for quantitative Norovirus-specific IgA, IgG, and IgM and total IgG.
- Vital signs will be obtained (temperature, BP, pulse, respiration and weight).
- Weekly Recall Packet will be administered (Table 5).
- Detailed PROs packet will be collected (Table 5).
- Collect concurrent medications.
- Assess for adverse events.
- Physical examination will be performed.
- Inquiry about hospitalization since last assessment.
- Pill count to assess adherence and left-over pills will be collected by study coordinator.

Instructions will be given to subjects for the Longitudinal Monitoring Phase diary (Diary 29-180 days) at the Day 28 visit.

If symptoms recur after discontinuing therapy, subjects can resume any therapy at the discretion of the principal investigator or his/her designee. Additional stool specimens should be collected prior to initiation of therapy for testing in the central lab, including stool for quantitative Norovirus PCR, sequencing and other pathogen testing when feasible.

e. Study Day 35 ± 3 days: Telephone Call for an update to health status and reminder for appointment
    - Collect concurrent medications.
    - Assess for adverse events.
    - Inquiry about hospitalization since last assessment.
    - Weekly Recall packet will be administered by the coordinator on the phone.

f. Day 53 ± 7 days-Telephone Call for an update to health status and reminder for appointment
    - Weekly Recall packet will be administered by the coordinator on the phone.
    - Appointment reminder for the next scheduled visit.

g. Study Day 60 ± 14 days: Option to do as a virtual visit or in person visit. Must be in person if medication not collected on day 28 visit.

- A pill count will be conducted if the patient did not complete dosing at the Day 28 visit.
- Review of completed diary pages.
- Subject to collect stool sample and store in freezer until day 120 or 180 study visit.
    - Samples of stool to be sent to the central laboratory are collected and processed (if in person visit):
        - Stool for quantitative Norovirus RT-PCR and sequencing.
        - Stool for other pathogen testing; stool will stored for later testing and this assay will not be performed for formed stool after Day 1.
        - Stool Norovirus-specific IgA, IgG, and IgM studies.
- Daily Diary and Detailed PROs packet will be collected (Table 5)
- Weekly Recall Packet will be administered (Table 5)
- Collect concurrent medications.
- Inquiry about hospitalization since last assessment.
- Assess for adverse events.

h. Study Day 113 ± 7 days-Telephone call for update to health status and reminder for appointment

- Weekly Recall packet will be administered by the coordinator on the phone.
- Appointment reminder for the next scheduled visit.

i. Study Day 120 ± 14 days: Option to do as a virtual visit or in person visit

- Review of completed diary pages.
- Subject to collect stool sample and store in freezer until day 180 study visit.
- Samples of stool to be sent to the central laboratory are collected and processed(if in person visit):
    - Stool for quantitative Norovirus RT-PCR and sequencing.
    - Stool for other pathogen testing; stool will stored for later testing and this assay will not be performed for formed stool after Day 1.
    - Stool Norovirus-specific IgA, IgG, and IgM studies.
- Daily Diary and Detailed PROs packet will be collected (see Table 5).
- Weekly Recall Packet will be administered (Table 5)
- Collect concurrent medications.
- Inquiry about hospitalizations since last assessment.

j. Study Day 173 ± 7 days-Telephone call for update to health status and reminder for appointment
   - Weekly Recall Packet will be administered by the coordinator on the phone.
   - Appointment reminder for the next scheduled visit.

k. Study Day 180 ± 14 days
   - Collection and review of completed diary pages.
   - WBC, TLC (Total Lymphocyte Count) Hb, Platelets (Derived from Complete Blood Count).
   - Creatinine, Alk Phos, AST, ALT, BUN, Bilirubin (Derived from Complete Metabolic Panel.
   - T Cell Subsets will be drawn unless CBC shows an ALC <100.
   - Samples of stool (collected at home or at clinic) to be sent to the central laboratory are collected and processed:
     - Stool for quantitative Norovirus RT-PCR and sequencing
     - Stool for other pathogen testing; stool will stored for later testing and this assay will not be performed for formed stool after Day 1.
     - Stool Norovirus-specific IgA, IgG, and IgM studies
   - Serum for quantitative Norovirus-specific IgA, IgG, and IgM and total IgG.
   - Vital signs (temperature, BP, pulse, respiration and weight) will be obtained.
   - Daily Diary and Detailed PROs packet will be collected (see Table 5).
   - Weekly Recall Packet will be administered (Table 5)
   - Collect concurrent medications.
   - Inquiry about hospitalizations since last assessment.

7.2 Follow-up

All subjects will be followed weekly while on study medication (visits on study Day 1, 7, 14, 21 and 28) and will be called 1 week after stopping medication to assess their clinical status and to assess for any adverse events. Subject will have laboratory assessments (WBC with total lymphocyte count), Hb, Platelets-derived from complete blood count and Creatinine, Alk Phos, AST, ALT, BUN, Bilirubin-derived from comprehensive metabolic panel) on study Day 1, 7, 28 and 180. Subjects will also be seen on study Day 60, 120 and 180 as part of the longitudinal monitoring phase of the study. If subjects develop recurrent diarrheal symptoms between visits, they should alert the study team, provide a sample of stool for central assessment and assessment locally. Likewise, if they develop adverse events between visits, they should alert the study team. More frequent evaluation can be done at the clinician's discretion. (*Please refer to Appendix A and Section 7 for details of study evaluations at each visit*)

7.3 Final Study Visit

The final study visit will take place on study Day 180 ± 14 days or if the subject withdraws from the study at an earlier time point. For any ongoing adverse event or serious adverse event, the subject should be followed until resolution of the event. (*Please refer to Appendix A and Section 7 for details of study evaluations at final visit*)

7.4 Early Termination Visit

If a subject is terminated from the study, they will be asked to return to the site as soon as possible after termination but no greater than 7 days after the decision to terminate them from the study. At this visit, the subject should return all unused study medication, their diary, and all procedures planned for Day 180 should be completed on this day. If termination is due to an adverse event or serious adverse event, the subject should be followed until resolution of the event with the consent from the subject. (*Please refer to Appendix A for details of study evaluations at each visit*)

7.5 Unscheduled Visit

If subjects develop recurrent diarrheal symptoms between visits, they should alert the study team, provide a sample of stool for central assessment and assessment locally. If the patient comes to the study site for an unscheduled visit, the patient should have vital signs, assessment for adverse events and review the patient diary and weekly PRO packet; laboratory assessments will only be collected if standard of care labs are ordered by the study team. Likewise, if they develop adverse events between visits, they should alert the study team. More frequent evaluation can be done at the clinician's discretion. *(Please refer to Appendix A for details of study evaluations at each visit*)

8 STUDY PROCEDURES/EVALUATIONS

8.1 Clinical Evaluations

1. Medical History
2. Physical Exam
3. Vital Signs including weight
4. Review of Concurrent Meds
5. Assessment for adverse events
6. Daily and Recall Diary (See Appendix C)
7. Weekly Patient Reported Outcomes Tool (See Appendix C)

To minimize missing diary data, we have added a recall diary protocol, administered by the coordinator at the appointment reminder call, which can be used to offset any missing daily diary data. In a subset of participants enrolled in Irritable Bowel Syndrome Outcome Study, IBSOS, prospective daily diary was aggregated across 7 days and served as the "gold standard" by which to compare end-of-period self-reported recall of symptoms over the past 7 days.[94] The participant groups' mean level of symptom severity based on weekly recall were within one point of each other for the core symptoms in this trial: diarrhea frequency and consistency [Bristol Stool Scale], urgency, and abdominal pain. If at all, participants were likely to over-estimate their symptoms on recall which would potentially favor our placebo condition. Because this prospective study, included participants who were already monitoring daily symptoms as part of a clinical trial, we recommend our participants continue to monitor daily during assessment periods in case the act of daily monitoring itself influences recall at one week. We will consider 80% compliance across the monitoring period acceptable [missing no more than 1.5/7 days per week over the 8 weeks]. While we will not exclude patients, who are below this compliance for any monitoring period, we will conduct a sensitivity analysis comparing those with few missing values to those with greater than 2 missing values per week.

A schematic of diary and PRO data collection is listed on the next page.

8.2 Laboratory Evaluations

8.2.1 Clinical Laboratory Evaluations

1. Complete Blood Count with Differential: This assay will be done in the individual site's laboratory using standard methodology. Normal ranges for the key components of the complete blood count (white blood cell count, neutrophil count, lymphocyte count, eosinophil count, monocyte, hemoglobin, hematocrit, mean corpuscular volume, and platelet count) will be obtained for each site. Assessment of these lab values will be conducted as

per described in Section 9.1 The Complete Blood Count will be collected as this generally considered standard of care by transplant centers, but only white blood cell count, hemoglobin and platelet count will be utilized for safety outcomes.
Comprehensive Metabolic Panel: This assay will be done in the individual site's laboratory using standard methodology. Normal ranges for the comprehensive metabolic panel (sodium, potassium, bicarbonate, chloride, blood urea nitrogen, creatinine, glucose, calcium, albumin, total protein, alkaline phosphatase, alanine amino-transferase, aspartate amino-transferase, and bilirubin) will be obtained for each site. Assessment of these lab values will be conducted as per described in Section 9.1. The Comprehensive Metabolic Panel will be collected as this is generally considered standard of care by transplant centers but only sodium, potassium, creatinine, alkaline phosphatase, alanine amino-transferase, aspartate amino-transferase, and bilirubin will be utilized for safety outcomes.

2. Urine Pregnancy Test: This assay will be done in the individual site's laboratory using standard methodology and must utilize an FDA-approved, cleared, or licensed test system.
3. T-Cell Subsets: This assay will be done in the individual site's laboratory using standard methodology to provide absolute number and % of CD3+, CD4+ and CD8+ cells.
4. Stool for Pathogen Screen: This assay will be performed in the Diagnostic Infectious Diseases Testing Laboratory (Cincinnati Children's Hospital Medical Center (CCHMC, Cincinnati, OH) which will be provided a 1-gram aliquot of stool from the Jiang Laboratory. Day 1 stool specimen and non-formed stool samples from subsequent visits will be screened for additional pathogens (Bacteria- Campylobacter (jejuni, coli and upsaliensis), Clostridium difficile (Toxin A/B), Plesiomonas shigelloides, Salmonella species, Yersinia enterocolitica, Vibrio species (parahaemolyticus, vulnificus and cholerae), Diarrheagenic E.coli/Shigella including, Enteroaggregative E. coli (EAEC), Enteropathogenic E. coli (EPEC), Enterotoxigenic E. coli (ETEC), Shiga-like toxin-producing E. coli (STEC), Shigella/Enteroinvasive E. coli (EIEC); Parasites- Cryptosporidium, Cyclospora cayetanensis, Entamoeba histolytica, Giardia lamblia; Viruses - Adenovirus F40/41, Astrovirus, Norovirus GI/GII, Rotavirus A, Sapovirus (I, II, IV and V)) using the FDA-cleared FilmArray system from BioFire Diagnostics (bioMerieux, Marcy l'Etoile, France). After collection in the stool hat, the subjects can wipe down and disinfect the outside of the container and place it in a bag for transport then refrigerate until they come to their visit with the research staff. The sample must have been produced within 24 hours of the visit and if it exceeds 24 hours it must be disposed. Subject would be encouraged to provide another sample within the visit window and if not, a rectal swab may be used for virus detection.

Table 5: Protocol PRO Completion Guide

Phase	**Treatment**					**Post-treatment monitoring**							**Unscheduled Visit**	**Early Termination Visit**
Assessment Time Point	Screen Enroll Day 1	Day 7 (±3 d)	Day 14₩ (±3 d)	Day 21₩ (±3d)	Day 28 (+3 d)	Day 35 (± 3d)	*Day 53 (±7d)	₩Day 60 (±14 d)	*Day 113 ±7d	₩Day 120 (±14 d)	*Day 173 ±7d	Day 180 (±14 d)		
Daily Diary		Days 1-7	Days 8-14	Days 15-21	Days 22-28			Days 29-60		Days 61-120		Days 121-180	X	Last week before term
Weekly Recall/ Phone Packet	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Detailed PROs Packet	X				X			X		X		X		X

Daily Diary: To be completed by subject daily. Diary 1-28 days; Diary 29-180 days See MOP for details.
Weekly Recall Packet: To be completed by study staff. See MOP for details.
Detailed PROs Packet: To be completed by study staff. This will include PROMIS and QOL Questionnaires. See MOP for details.
*Phone call-Appointment reminder and Weekly Recall Packet to be completed
₩Can be Virtual Visits

8.2.2 Special Assays or Procedures

1. Stool for Norovirus Assays: These assays will be performed in the Jiang Laboratory (CCHMC, Cincinnati, OH). Bulk stool samples are collected from subjects and aliquoted on site into cryovials. Aliquots are frozen at <-70°C before shipment to the Jiang Lab. One-gram bulk stool aliquots are necessary for the following assays.
   a. Norovirus Quantitative RT-PCR (qRT-PCR): 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 in triplicate using the TaqMan Fast Virus 1-Step Master Mix (Applied Biosystems). GI NoVs are amplified with forward primer – QNIF4 5'CGC TGG ATG CGN TTC CAT, reverse primer – NV1LCR 5'CCT TAG ACG CCA TCA TTT AC, and probe – NV1LCpr 5'VIC-TGG ACA GGA GAY CGC RAT CT-MGBNFQ, while GII NoVs are amplified 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). All samples will be compared among each other, as well as against known GI and GII positive controls for relative Ct values.

      Stool samples will be processed for extraction of norovirus RNAs. RT-PCR will be performed via a standard procedure using primers targeting the RNA-dependent RNA polymerase gene of norovirus genome. This will result in a 319-nucleotide fragment as PCR products. PCRs will contain 40 cycles of amplifications; a positive and negative control will be included to make sure PCR being working. As explained above, stool sampling will be performed according to an SOP; there is no approaches at the laboratory level to account for sampling error. The PCR product of norovirus genome fragment will be sequenced at CCHMC's DNA Sequencing Core Facility equipped with state-of-the-art capillary DNA analyzers (Sanger Sequencing).
   b. Norovirus Genotyping: Once NoV-positive samples have been identified by qRT-PCR, routine RT-PCR and sequencing can be employed to identify genotype. This amplification uses a set of primers (p289H,I/p290H,I,J,K) designed to detect all NoV genotypes. PCR products are run on an agarose gel and visible bands at the correct size are cut from the gel, purified, and sequenced. Sequences are compared to published NoV sequences with known genotypes downloaded from GenBank to determine genotype.
   c. Norovirus Phylogenetic Analysis: After sequencing RT-PCR products from subjects over the course of the study to determine genotype, as described above, sequences from the same subject over time will be compared to identify mutations. Various analyses can be performed to assess sequences changes over time.

2. Stool for Norovirus-Specific Immunoglobulin Studies: These assays will be performed in the Jiang Laboratory (CCHMC, Cincinnati, OH). Bulk stool samples are collected from subjects and aliquoted on site into cryovials. Aliquots are frozen at <-70°C before shipment to the Jiang Lab. One-gram bulk stool aliquots are necessary for the following assays. For stool Norovirus-specific IgA, IgM and IgG studies, a 96-well microtiter plates are coated with rabbit antibody specific to Noroviruses overnight at 4°C. Following a wash the plates are blocked with 5% nonfat dry milk. For Norovirus-specific Igs, purified Norovirus P-particles (PP) are then added. After incubation 1 hour at 37°C, dilutions of subject 10% stool suspensions are added and the plates are incubated at 37°C for 1 hour. Horseradish peroxidase conjugated goat anti-human Ig 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 stool.
3. Stool Pathogen Screen: The Stool Pathogen Screen will utilize the BioFire FilmArray Gastrointestinal Panel to screen for a range of pathogens (*Campylobacter* (*C. jejuni/C. coli/C. upsaliensis*),*Clostridium difficile* (*C. difficile*) toxin A/B, *Plesiomonas shigelloides, Salmonella, Vibrio* (*V. parahaemolyticus/V. vulnificus/ V. cholerae*), including specific identification of *Vibrio cholerae, Yersinia enterocolitica,* Enteroaggregative *Escherichia coli* (EAEC), Enteropathogenic *Escherichia coli* (EPEC), Enterotoxigenic *Escherichia coli* (ETEC) *lt/st,* Shiga-like toxin-producing *Escherichia coli* (STEC) *stx1/stx2* (including specific identification of the *E. coli* O157serogroup within STEC), *Shigella*/ Enteroinvasive *Escherichia coli* (EIEC), *Cryptosporidium, Cyclospora cayetanensis, Entamoeba histolytica, Giardia lamblia* (also known as *G. intestinalis* and *G. duodenalis*), Adenovirus F 40/41, Astrovirus, Norovirus GI/GII, Rotavirus A, Sapovirus (Genogroups I, II, IV, and V)). The samples are placed in Cary-Blair. Test is performed according to manufacturer's instructions. Once completed, the results are reviewed for internal controls and possible pathogen detection. The internal controls must be positive for the run to pass. If controls fail, the samples is retested with a new pouch. Any unusual pathogens detected will be brought to the attention of the Director and study PI.
4. Serum for Immunoglobulin Assays: These assays will be performed in the Jiang Laboratory (CCHMC, Cincinnati, OH). For these assays, at least 4, 1 mL serum aliquots are required. Whole blood is collected in 10 mL SST tubes and serum is processed and aliquoted into cryovials at the sites and frozen at <-70°C before shipment to the Jiang Lab.
   a. Quantitative Total IgG Level: Total IgG levels are also measured using a similar EIA method. Instead of coating Norovirus-specific antibodies to the microtiter plates, anti-IgG antibodies are used for the initial coating before addition of subject serum samples. Similar to above, horseradish peroxidase conjugated anti-human Ig antiserum is added to each plate before TMB substrate and phosphoric acid addition and measurement. Known

standard concentrations of total IgG can be included for quantitative assessment of subject samples compared to the standards.

b. Norovirus-Specific Serum IgA, IgM and IgG: 96-well microtiter plates are coated with rabbit antibody specific to Noroviruses overnight at 4°C. Following a wash the plates are blocked with 5% nonfat dry milk. For Norovirus-specific Igs, purified Norovirus P-particles (PP) are then added. After incubation 1 hour at 37°C, subject serum samples are added and the plates are incubated at 37°C for 1 hour. Horseradish peroxidase conjugated goat anti-human Ig 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.

For the NoV-specific ELISAs, they will be performed for subjects after the sample genotyping has been completed. Since we are interested in the antibody responses in each subject, the antigens in the ELISAs will match their genotypes. The ELISA SOPs (see MOP) gives the example of our NoV GII.4 P-domain protein as the antigen that we make in-house but also states that other genotypes may be used as long as the protein concentration has already been determined for the antigen.

5. Buccal Swab/Saliva for Secretor Status Phenotype and Genotype: These assays will be performed in the Jiang Laboratory (CCHMC, Cincinnati, OH). 5-10 mL of saliva will be collected in a sterile cup and aliquot into 5 1mL tubes which can then be frozen at <-70°C C before being shipped to the Jiang Lab. Buccal swab will be collected by rubbing the oral swab on the cheeks and gums. This should provide enough buccal cells for the analysis. Swabs are stored in 1 mL distilled water in the swab container and can be sealed then frozen at <-70°C before shipment to the Jiang Lab.

   Both phenotypic and genotypic evaluation will be performed to compare results of the two methods. In the case of discordant results, for this trial, the results from the phenotypic method will be used to classify secretor status.

   a. Secretor Status Phenotyping: The secretor status of individual subjects will be determined based on the detection of histo-blood group antigens (HBGAs) for the secretor antigens (Leb, Ley, H types) vs. the non-secretor antigens (Lea and Lex) in their saliva. In this assay, boiled human saliva samples are diluted and used to coat wells of a 96-well EIA 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 HRP-conjugated anti-mouse antibody of the correct isotope determines if a sample is positive for a particular HBGA.

   b. Secretor Status Genotyping: A FUT2 genotyping method using sequence specific primers-PCR (SSP-PCR) is based on single nucleotide polymorphisms (SNPs) of the FUT2 gene. Primers covering three major mutations are synthesized [wild-type 385A (5'-

AGGAGGAATACCGCCACAT), mutated 385T (5'-GAGGAGGAATACCGCCACT), wild-type 428G (5'-GCTACCCCTGCTCCTGG), mutated 428A (5'-CGGCTACCCCTGCTCCTA), wild-type 571C (5'-TAGGGGTCCATGTTCGCC), and mutated 571T (5'-GTAGGGGTCCATGTTCGCT)]. An antisense primer (5'-GGCTGCCTCTGGCTTAAAG) is paired with FUT2 wild-type and mutated sense primers. DNA is extracted from buccal cells in saliva samples. DNA amplification is carried out using the following program: 95°C for 2 min, 10 cycles of 95°C for 20 sec, 66°C for 1 min, and 70°C for 1 min; then 20 cycles of 95°C for 20 sec, 62°C for 1 min, and 72°C for 1 min; and a final extension at 72°C for 5 min. The human growth hormone gene is used as PCR internal control.

6. Nitazoxanide Blood Levels for Pharmacokinetic Assessments: First 20% of the subjects who consent (20% convenience sample) will be asked to provide PK samples. After 20% sample requirements have been met, NU team will notify sites as to no longer ask subject to participate in providing this sample.

   All samples are de-identified with only study assigned numbers. This assay will be conducted by SGS Biopharma SA, Wavre, Belgium who is the only company that assesses nitazoxanide blood levels. Whole blood sample will be centrifuged (At 1500g during 10 minutes at 4°C) within maximum 30 min after blood collection. Blood collection has to be performed in Li Hep polyproprylene collection tube. 2 aliquots of around 400µL of plasma will be sufficient for tizoxanide determination. A maximum of 486 days has been validated for the long term storage of plasma samples below -20°C. Therefore, it's recommended to ship samples to the bioanalytical laboratory after maximum 400 days of storage to perform the analyses of tizoxanide and its glucuronide. These will be shipped first to the Jiang lab and then onto SGS for testing per Romark, L.C. approved protocol.

8.2.3 Specimen Preparation, Handling, and Shipping

Specific collection and handling of assays will be as below (Table 6). Local laboratories should analyze specimens on the day of specimen collection.

Specific instructions will be provided to subjects about specimen handling at home and transportation to the study site (outlined in the MOP). The sites may call the subjects the night before the in-person visit to remind the subjects to bring in the samples with them.

Table 6: Specimen Collection, Preparation, Processing and Storage

Sample Collection Schedule for Assays	Objective	Study Day	Collection Tube	Processed Assayed*	Processing / tubes needed	Use as	Storage
Blood for Serum							
Ig studies (NoV-specific IgA, IgG, and IgM and total IgG)	Exploratory	1, 28	10mL Gold tube	Sites Jiang Lab	1mL aliquots in 2mL vials 2 x 1mL required	Frozen	Samples will be shipped to Jiang Lab.
	Future Use	180	10mL Gold tube	Sites Jiang Lab	1mL aliquots in 2mL vials 2 x 1mL required	Frozen	Samples will be shipped to Jiang Lab
Saliva							
Saliva for Secretor Status Phenotype and Genotype	Exploratory	1	Sterile Cup	Sites Jiang Lab	1mL aliquots in 2mL vials 5 x 1mL required	Frozen	All samples will be shipped to Jiang Lab.
Buccal Swab for Secretor Status Phenotype and Genotype	Exploratory	1	1 swab in 1mL distilled water	No processing Jiang Lab	1 swab in 1mL distilled water	Frozen	All samples will be shipped to Jiang Lab.
Stool							
NoV Viral Load by qRT-PCR	Secondary	1, 7, 14, 21, 28, 60, 120, 180, and with recurrent diarrhea	1g aliquots	Sites Jiang Lab	1g aliquots 2 x 1g required	Frozen	Samples will be shipped to Jiang Lab.
Ig studies (NoV-specific IgA, IgG, and IgM	Exploratory	1, 28, 60	1g aliquots	Sites Jiang Lab	1g aliquots 2 x 1g required	Frozen	Samples will be shipped to Jiang Lab.
	Future Use	14, 120, 180	1g aliquots	Sites Jiang Lab	1g aliquots 2 x 1g required	Frozen	Shipped to Jiang Lab
NoV RT-PCR and Sequencing	Exploratory	1, 28, 60	1g aliquots	Sites Jiang Lab	1g aliquots 1 x 1g required	Frozen	Samples will be shipped to Jiang Lab.
	Future Use	7, 14, 21, 120, 180, and with recurrent diarrhea	1g aliquots	Sites Jiang Lab	1g aliquots 1 x 1g required	Frozen	Samples will be shipped to Jiang Lab
Pathogen Screen (BioFire)	Exploratory	1, 180 (all subjects) also 7, 14, 21, 28, 60, 120, and with recurrent diarrhea if unformed stool on study day	1g aliquots	Sites Mortensen Lab	1g aliquots 1 x 1g required	Frozen	Samples will be shipped to Jiang Lab-will provide stool aliquots to Mortensen Lab (3333 Burnet Ave, ML 1010, Cincinnati, OH 45229) as requested when ready to perform assay.
Backup Specimen	Exploratory and Future Use	1, 7, 14, 21, 28, 60, 120, 180, and with recurrent diarrhea	1g aliquots	Sites Jiang Lab	1g aliquots 3 x 1g required	Frozen	Samples will be shipped to Jiang Lab

Initial sample collection tubes, except for the buccal swab, will be labeled with the study number, subject ID number, and date obtained. The buccal swab initial collection tube and all processed sample aliquots to be tested in the central laboratory will be barcoded.

The central laboratory for this study will be Dr. Xi Jason Jiang's laboratory at Cincinnati Children's Hospital Medical Center. Northwestern will provide the sites with key required study consumables (e.g. blood and stool collection tubes), shipping containers and FedEx pre-paid shipping labels for all specimens being tested in Dr. Jiang's laboratory

Jiang Lab
Attn: Ming Tan, PhD
Division of Infectious Diseases, S8.537
Building S, Receiving Dock 1
240 Albert Sabin Way
Cincinnati, OH 45229

In addition, blood collected for the PK testing performed will be stored and sent to SGS Biopharma SA, Wavre, Belgium for testing; samples for PK testing will be sent to SGS Biopharma once per year.

Frozen samples will be batched and shipments to the central laboratory will occur at minimum every other month after communication with Dr. Jiang's study coordinator. All residual specimens will remain in the Jiang Laboratory until the end of the study. At this time unused sample aliquots will be returned to the NUCTC laboratory for banking. Detailed instructions for sample preparation, handling, storage and shipment are included in the protocol-specific Manual of Procedures.

9 ASSESSMENT OF SAFETY

9.1 Specification of Safety Parameters

For HSCT, the rate of survival and hospitalization is greatly dependent upon the underlying malignancy, the type of stem cell transplantation and the stage of disease at the time of transplantation. As such, a single mortality rate is challenging to report. The 1 year survival of autologous HSCT for multiple myeloma is 87.1% (95% CI 84.8-89.1%); 1 year survival for acute myeloid leukemia is 75.4% (95%CI 70.2-79.8%), 72.5% (95%CI 70.6-74.2%), and 64.8% (95%CI 63.1-66.3%) for autologous, allogeneic (HLA-identical), and allogeneic (matched unrelated), respectively.[93]

For SOT, the rate of survival and hospitalization is greatly dependent on the specific organ, the indication for transplantation and the type of organ transplanted (living vs. deceased donor). For patients treated at Northwestern, the 1 year survival rate is 98.3% for kidney transplant, 87.2% for liver transplant and 95.7% for pancreas transplant. Admission data is typically measured at 30 days for transplant and greatly falls off after 30 days. At Northwestern, the rate of 30-day hospitalization is 28% and 13% for deceased and living donor kidney transplantation and 31% and 54% for deceased and living donor kidney transplantation.

The safety of nitazoxanide will be assessed by the frequency of protocol specified unsolicited clinical adverse events, laboratory adverse events and serious adverse events. We observe the following safety parameters: Laboratory values, clinical assessments and subject complaints as collected on the subject diary.

The safety follow-up period for nitazoxanide will be through day 60 and will include telephone based assessment on Day 35 and laboratory testing performed on Day 60. Any abnormal labs on Day 60 will be followed based at the discretion of the study site physician until value returns to the normal range or baseline level for the subject.

9.2 Methods and Timing for Assessing, Recording, and Analyzing Safety Parameters

9.2.1 Adverse Events

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

Unsolicited clinical AEs are those non serious AEs that are **not** being collected as clinical outcome measures. For this study, unsolicited clinical AEs to be captured on the appropriate case report form, will be only those clinical AEs that meet either of the following criteria through 60 days:

- Result in modification in the administration of the study drug (nitazoxanide or placebo).
- Result in discontinuation of the study drug (nitazoxanide or placebo).

The following solicited safety laboratory parameters will be assessed on Day 7, 28 and 60: WBC, Hemoglobin, Platelet count, Creatinine, Alkaline Phosphatase, ALT, AST, BUN and Bilirubin. Additional laboratory parameters performed as part of the CBC and complete metabolic panel results need to be recorded in the source document with the evaluation of the site physician. Those additional laboratory value will need to be reported as AE only if clinically significant and not attributable to Norovirus infection or underlying SOT/HSCT medical conditions.

Abnormal values observed in the clinical laboratory assessment that are conducted by the clinical team outside the study visits, will be reported as AE only if the site physician assess that the laboratory value is clinically significant and not attributable to Norovirus infection or underlying SOT/HSCT medical conditions. The evaluation should be recorded in the source documents.

Laboratory AEs will be reported as defined in section 9.2.3.

Information to be collected for these unsolicited clinical AEs and laboratory AEs includes event description, time of onset, clinician's assessment of severity, relationship to study product (assessed only by those with the training and authority to make a diagnosis- a licensed study physician listed on the Form FDA 1572 as the site principal investigator or sub-investigator), and time of resolution/stabilization of the event. These events occurring while on study must be documented appropriately regardless of relationship will be followed to adequate resolution.

Any medical condition that is present at the time that the subject is screened should be considered as baseline and not reported as an AE. However, if it deteriorates at any time during the study, it should be recorded as an AE as defined above.

Severity of Event: Unsolicited clinical AEs (as defined above) and all protocol specified SAEs will be assigned the severity level of Grade 3. Laboratory AEs will be assessed by the clinician using a protocol defined grading system (Appendix B).

Relationship to Study Products: The clinician's assessment of an AE's relationship to test article (study drug) is part of the documentation process, but it is not a factor in determining what is or is not reported in the study. If there is any doubt as to whether a clinical observation is an AE, the event should be reported. AEs must have their relationship to study product assessed using the terms: related or not related. 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.

9.2.2 Serious Adverse Events

Hospitalizations are being collected as an outcome measure for the secondary objective and therefore for this study will not be reported as SAEs.

An adverse event or suspected adverse reaction is considered "serious" if, in the view of either the site principal investigator or sponsor, it results in any of the following outcomes through Day 60:

- Death,
- A life-threatening adverse event*,
- A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions, or
- A congenital anomaly or birth defect,
- Important medical events that may not result in death, be life threatening, or require hospitalization, 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 investigator or sponsor, its occurrence places the patient or subject at immediate risk of death. It does not include an adverse event that, had it occurred in a more severe form, might have caused death.*

Unexpected: An adverse reaction, the nature or severity of which is not consistent with the applicable product information (e.g. IB for an unapproved investigational medicinal product). SAEs will be:

- Assessed by a licensed study physician listed on the Form FDA 1572 as the site principal investigator or sub-investigator.
- Recorded on the appropriate SAE form and CRF.
- Followed through resolution or stabilization by a study physician licensed to make medical diagnoses and listed on the Form FDA 1572 as the site principal investigator or sub-investigator.
- Reviewed and evaluated by the DSMB (periodic review), DMID, and the IRB.

9.2.3 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.

The criteria for an abnormal laboratory test finding being classified as an AE are any of the following and will be documented on the AE form:

- Test result is associated with a sign or symptom, and/or
- Test result requires additional diagnostic testing, and/or
- Test result requires a medical or surgical intervention, and/or
- Test result leads to a change in study dosing outside of the protocol defined dosing or discontinuation from the study treatment, and/or
- Test result requires significant additional treatment, i.e. addition of new medication, significant increase in dose of current medication

Baseline clinical labs performed on Day 1 (screening/enrollment visit) might fall outside of the normal range. Laboratory values in all subsequent visits for subjects in which baseline clinical labs results fall outside the normal range will be considered adverse events only if there is a worsening in the grading of the event (e.g., value that on screening falls within grade 1, and subsequently on grade 2 will be considered AE; value that on both screening and on subsequent testing fall within grade 2 will not be considered AE).

For those clinical testing that performed on Day 1 and falls within grade 3, for all subsequent testing that also falls within grade 3, the clinician will need to assess and document this assessment on the source documents whether there is a clinically significant worsening of the value. Only those laboratory value that judged to have a clinically significant worsening and not attributable to Norovirus infection or underlying SOT/HSCT medical conditions will be reported as AEs.

The protocol defined grading system is used to determine the cut off point for reporting. As such, laboratory results that are abnormal according to the local laboratory reference range but not considered a Grade 1 abnormality, will not be considered laboratory AEs. Those events will be followed-up clinically at the discretion of the study site physician.

9.3 Reporting Procedures

9.3.1 Serious Adverse Events

Any AE that meets a protocol-specified serious criterion (see section 9.2.2) through Day 60 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 Phone Number: 1-800-275-7619 (US) or 1-301-897-1710 (outside US)
SAE Email Address: PVG@dmidcroms.com

SAEs will be followed until satisfactory resolution or until the PI or Co-Investigator deems the event to be chronic or the subject to be stable

In addition to the SAE form, selected SAE data fields must also be entered into AdvantageEDC^{SM}. 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.

SAEs will be reported to the IRB in accordance with IRB policy.

Note: Hospitalizations are being collected as an outcome measure for the secondary objective and therefore for this study will not be reported as SAEs.

9.3.2 Regulatory Reporting for Studies Conducted Under DMID-Sponsored IND

Following notification from the site principal investigator or appropriate sub-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 site principal investigators (i.e., all principal 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.

9.3.3 Reporting of Pregnancy

Pregnancies occurring in study subjects will be reported via AdvantageEDC^{SM} on the Report form. No further study drug will be administered to pregnant subjects, but with the subject's permission all protocol-required samples will be obtained, and the subject will continue to be followed for safety for the duration of this trial. Efforts will be made to follow all pregnancies reported during the course of this trial to pregnancy outcome pending the subject's permission.

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

Potentially study drug-related AEs and SAEs will be followed from the time of the first study procedure through 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. Follow-up procedures, evaluations, and outcomes will be recorded on the appropriate data collection form.

9.5 Halting Rules

Further enrollment will be halted for DSMB review/recommendation if any of the following are reported through Day 60:

- Two or more subjects experience a study product-related SAE.
- ≥4 subjects experience the same severe (Grade 3) study product-related unsolicited AE until 80 subjects enrolled or ≥5% or more subjects experience the same severe (Grade 3) study product-related unsolicited AE after 80 subjects have been enrolled.
- ≥4 subjects experience the same severe (Grade 3) study product-related safety laboratory AE until 80 subjects enrolled or ≥5% or more subjects experience the same severe (Grade 3) study product-related safety laboratory AE after 80 subjects have been enrolled.

Further enrollment will be halted for DSMB review/recommendation if the ratio of each clinical adverse outcome incidence rate per subject between the study arms is two or higher through Day 180:

- Death: Minimum of 4 or more subjects experience death in all study arms.
- Hospitalization: Minimum of 10 or more subjects experience hospitalization in all study arms.
- Graft rejection: Minimum of 5 or more subjects experience graft rejection in all study arms.
- Graft loss: Minimum of 5 or more subjects experience graft loss in all study arms
- Renal insufficiency, defined as a 20% or greater increase in serum creatinine relative to screening/enrollment/baseline value: Minimum of 10 or more subjects experience renal insufficiency in all study arms.

DMID retains the authority to suspend additional enrollment and study interventions/ administration of study product during the entire study, as applicable.

9.6 Safety Oversight (ISM plus SMC or DSMB)

9.6.1 Independent Safety Monitor

For this clinical trial, an ISM is not required. However, at each participating site, upon DMID Medical Monitor request, the principal investigator (PI) will identify a physician with relevant expertise, to act as a Secondary Medical Assessor (SMA). The SMA will examine a subject and/or medical records and provide a medical assessment (or second medical opinion) to the DMID of the safety event in question. The PI will send to the DMID MM, a summary of the event and include the PI and SMA assessments.

Note: In the case that DMID has requested this type of evaluation multiple times, DMID may request the site(s) identify a SMA to assist DMID with safety oversight.

9.6.2 Data and Safety Monitoring Board (DSMB)

Safety oversight will be conducted by a DSMB that is an independent group of experts that monitors subject safety and advises DMID. The DSMB members will be separate and independent of study personnel participating in this trial and should not have scientific, financial or other conflict of interest related to this trial. The DSMB will consist of members with appropriate expertise to contribute to the interpretation of the data from this trial.

The DSMB will operate under the rules of a DMID-approved charter that will be written at the organizational meeting of the DSMB. Procedures for DSMB reviews/meetings will be defined in the charter. Reports may include enrollment and demographic information, medical history, concomitant medications, physical assessments, clinical laboratory values, dosing compliance, and solicited and unsolicited AE/SAEs. The DSMB will review SAEs on a regular basis and ad hoc during this trial. The DMID Medical Monitor and the ISM (as deemed necessary) will be responsible for reviewing SAEs in real time.

The DSMB will review and discuss study data:

- At the following enrollment milestones with the relevant percentage of patients completing Day 60 of the study: 25% (N=40), 50% (N=80), 75% (N=120);the DSMB will review available safety data through Day 60 and Norovirus infection clinical adverse outcome through Day 180 and available clinical and virologic efficacy data and study product nitazoxanide retreatment data.
- Final review meeting: 6 to 8 months after clinical database lock to review the cumulative unblinded safety and efficacy data for the study. The data will be provided in a standard summary format. The DSMB may be asked to provide recommendations in response to questions posed by DMID.
- Ad hoc review: may be in response to a safety issue such as a halting rule being met.

(Enrollment will be monitored to ensure there is a DSMB review each year of the study during active enrollment. In the case one of the enrollment milestones has not be met and a review has not occurred within the previous 12 months, DMID may request the DSMB review the study for safety.)

The DSMB may also be convened for an ad hoc meeting. An Ad hoc meeting is an unplanned meeting that is called for a specific purpose such as when a study halting rule is met. The meeting can be requested by any party with the responsibility of overseeing the trial (such as the PI, DSMB, DMID, industry collaborator). In the case of an ad hoc meeting, the DSMB may request special reports on an as-needed basis.

If the study is discontinued, subjects will not receive study product and, follow-up visits for safety would continue.

Additional data may be requested by the DSMB, and interim statistical reports may be generated as deemed necessary and appropriate by DMID. The DSMB may receive data in aggregate and presented by treatment arm. The DSMB may also be provided with expected and observed rates of the expected AEs in an unblinded fashion, and may request the treatment assignment be unblinded for an individual subject if required for safety assessment. The DSMB will review grouped and unblinded data in the closed session only. As an outcome of each review/meeting, the DSMB will make a recommendation as to the advisability of proceeding with study investigational product (as applicable), and to continue, modify, or terminate this trial.

10 CLINICAL MONITORING

10.1 Site Monitoring Plan

Site monitoring is conducted to ensure that the human subject 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. 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, CRFs, source data (paper and electronic), audit trails, ICFs, medical and laboratory reports, and protocol and GCP compliance. Site monitors will have access to the study 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 document visit findings and discussions.

11 STATISTICAL CONSIDERATIONS

11.1 Study Hypotheses

A formal hypothesis test is planned to compare nitazoxanide to placebo with respect to the primary efficacy outcome measure. The time from randomization until clinical resolution of Norovirus for at least 48 hours through 180 days will be compared between the two study arms.

The null hypothesis is that there is no difference in time until clinical resolution between study arms, with a two-sided alternative. The test for primary efficacy hypothesis will be conducted using a Log-Rank test at the 5% two-sided level of significance.

11.2 Sample Size Considerations

Sample size calculations assume a 50% reduction in the median duration of diarrhea for the nitazoxanide-treated group compared to those receiving placebo. The median diarrhea duration for the placebo group is assumed to be 135 days, while the median diarrhea duration for the treatment group is assumed to be 67.5 days. Given these assumptions, a sample size of 64 subjects in each arm (128 total) is required to achieve 90% power using the Log-Rank test. Accounting for a 20% attrition rate, final sample size will be 80 in each arm (160 total).

Though discouraged, the protocol allows for subjects in either study group to receive additional nitazoxanide after 28 days. Under the assumptions of treatment efficacy, additional treatment received by placebo subjects would reduce the median duration of symptoms for the placebo group to be closer to that of the nitazoxanide-treated group. It is assumed that subjects who would have the worst study outcomes (longest time to clinical resolution of diarrhea) will be the most likely to receive additional treatment. Subjects receiving additional treatment will be imputed as having the worst outcome (time to resolution censored at 180 days). Accounting for this imputation method, the power attained for the primary comparison was computed via simulation (R version 3.0.3). Estimated power is displayed in the following table, varying the proportion of subjects in each study group who receive additional treatment

		Proportion Receiving Additional Treatment in the Placebo Group						
		0%	5%	10%	15%	20%	25%	30%
Proportion re-treated in the nitazoxanide Group	0%	91.3%	91.6%	91.7%	91.6%	91.8%	92.0%	92.3%
	5%	91.4%	91.5%	91.6%	91.2%	91.2%	91.8%	92.5%
	10%	-	91.5%	92.0%	91.2%	91.8%	91.2%	92.2%
	15%	-	-	90.7%	90.7%	91.0%	91.6%	91.8%
	20%	-	-	-	87.3%	87.1%	87.0%	87.7%
	25%	-	-	-	-	74.0%	73.9%	75.2%
	30%	-	-	-	-	-	40.0%	40.9%

For example, if 15% of placebo subjects receive nitazoxanide, and 5-10% of subjects in the nitazoxanide-treated group receive additional treatment, the power is estimated to be 91.2%. Note that implausible scenarios (where substantially more nitazoxanide subjects receive additional treatment than do placebo subjects) have been omitted from the table.

11.3 Planned Interim Analyses

11.3.1 Safety Review

As described in Section 9.6, the DSMB will meet and review safety data at regular enrollment milestones. The study will be monitored to determine if any of the safety halting rules described in Section 9.5 is met. The halting rules do not utilize any statistical criteria and no formal hypothesis testing is planned to occur for the safety reviews.

11.3.2 Efficacy Review

The DSMB will review available clinical and virologic efficacy data, however no formal hypothesis testing is planned.

11.4 Final Analysis Plan

11.4.1 General Principles

The SDCC will perform all analyses after final data lock. Tabulations will be used extensively to summarize the data. Summary statistics displayed for continuous data will include measures of central tendency such as the mean or median, and measures of dispersion such as the standard deviation or inter-quartile range. Summary statistics for discrete data will include absolute and relative frequencies and confidence intervals where appropriate.

In presenting statistical inferences, the statistical claim (i.e. null and alternative hypotheses) will be clearly stated, the method used for hypothesis testing will be described and appropriately referenced, and the assumptions underlying the methods will be validated.

More details of the analyses to be performed will be included in a separate Statistical Analysis Plan.

11.4.2 Analysis Populations

The safety population (grouped by actual treatment received) will include all subjects who are randomized, have received at least one dose of study treatment and have at least one safety assessment.

The modified intent-to-treat (mITT) population includes all randomized subjects who received at least one dose of assigned study drug. Analyses are performed according to randomized treatment assignment, not the treatment received.

The per-protocol (PP) population will include all subjects who met all inclusion/exclusion criteria, complied with the assigned study product, complied with the daily diary, and were followed up until resolution of symptoms, or completed 180 days without resolution of symptoms.

A subject is considered to be compliant with the assigned study product if he/she takes at least 75% of the scheduled doses through 28 days, and does not receive additional nitazoxanide before their primary outcome is observed.

A subject is considered to be compliant with the daily diary if he/she is missing no more than 1.5/7 days per week over the 8 weeks.

11.4.3 Efficacy Analyses

11.4.3.1 Analysis of Primary Outcome Measure

The primary efficacy outcome measure is the time from randomization to initial clinical resolution of Norovirus for at least 48 hours, assessed through 180 days, in each study arm. Clinical resolution will be assessed from the daily diary by the subject and will be defined as:

a. Cessation of vomiting and
b. No stools classified by the Bristol Stool Chart as diarrhea (Type 6 or 7)

The primary efficacy outcome measure will be assessed in the mITT population. Subjects who receive additional treatment with nitazoxanide other than their assigned study product will be considered censored at 180 days. To assess the effect of the choice of analysis population and additional treatment, the analysis will be repeated as a secondary analysis in the PP population. Sensitivity analyses may be performed to further assess the effect of additional treatment. The details of any sensitivity analyses will be detailed in the Statistical Analysis Plan.

The null hypothesis that the time to first clinical resolution of Norovirus is the same in both treatment arms will be tested using a stratified Log-Rank test to take into account the stratification at randomization. Estimates for median time to resolution and corresponding 95% confidence intervals will be reported for each treatment arm.

We will consider 80% compliance across the monitoring period acceptable [missing no more than 1.5/7 days per week over an 8 week period]. While we will not exclude patients, who are below this compliance for any monitoring period, we will conduct a sensitivity analysis comparing those with few missing values to those with 2 or more missing values per week.

With respect to missing data for the primary endpoint, the primary analysis will be handled as follows:

- Data that are missing after a subject has reached clinical resolution will not affect the primary time to event analysis and therefore will be ignored

- Subjects who drop out, are lost to follow-up, withdraw consent, or otherwise stop completing daily diary entries before clinical resolution will be censored on the day of their last completed diary

- Subjects with a gap in outcome data prior to clinical resolution will be handled depending on their last completed diary entry and their next completed diary entry:

    - If the last completed diary entry and the next completed diary entry have the same outcome (i.e., they are still symptomatic on both days or they have no symptoms on both days), then the gap will be imputed with the same outcome

    - If this imputation results in the subject being defined as having clinical resolution during this gap, their time to resolution will be the median of the interval

- If the last completed diary entry and the next completed diary entry have different outcomes (i.e., the last one is symptomatic and the next one is asymptomatic, or vice versa), then the gap will be imputed with a symptomatic outcome (no resolution)

The above method will be considered primary and additional methods such as worst case scenario or best case scenario may be explored as supportive analyses to assess robustness of results.

11.4.3.2 Analysis of Secondary Efficacy Outcome Measures

All secondary efficacy outcome measures will be assessed in the mITT and PP populations separately.

The secondary efficacy outcome measures are as follows:

1. The time from the randomization to first negative viral load through 180 days.
2. The change in viral titer between Day 1 and Day 180.

Secondary outcome measure 1 will be assessed through Kaplan-Meier curves along with results from a Log-Rank test to compare the time from randomization to first negative viral load between the nitazoxanide treated group and the placebo group.

Secondary outcome measure 2 will be assessed using analysis of covariance (ANCOVA) adjusting for baseline viral titer.

11.4.3.3 Sensitivity Analysis of Primary and Secondary Efficacy Outcome Measures

As a sensitivity analysis, all primary and secondary efficacy analyses will be conducted among the subgroup of subjects who do not report chromaturia (discoloration of urine) based on unsolicited self-reporting, in order to help determine if knowledge of this side effect may have impacted these outcomes.

11.4.4 Safety Analyses

Unsolicited AEs will be coded by MedDRA for preferred term and system organ class. The proportion of subjects and exact 95% confidence intervals of AEs in aggregate, as well as by MedDRA categories, will be computed. The number of SAEs will be reported by a detailed listing showing the type, MedDRA coding, relevant dates (treatment dosing dates and AE onset and resolution dates), severity, relatedness, and outcome for each event.

11.4.4.1 Analysis of Secondary Safety Outcome Measures

Safety outcome measures will be analyzed in the safety analysis population.

The safety outcome measures are as follows:

1. Incidence of unsolicited non-serious adverse events through 60 days.
2. Incidence of laboratory adverse events (WBC, Hemoglobin, Platelet count, Creatinine, Alkaline Phosphatase, ALT, AST, BUN, and Bilirubin) through 60 days.
3. Incidence of protocol-specified serious adverse events (see section 9.2.3 for details) through 60 days.
4. Incidence of hospitalization through 60 days.

For all safety outcome measures above, the proportion of subjects with events will be presented by treatment arm and overall. Point estimates for proportions and difference in proportions along with the corresponding 95% confidence intervals will be reported. A Barnard's exact test at the 5% two-sided level of significance level will be used for the comparisons of proportions between treatment arms.

11.4.5 Exploratory Analyses

The exploratory outcome measures are as follows:

Clinical Improvement;

1. Time from randomization to initial clinical improvement in Norovirus disease through 28 and 180 days for at least 48 hours, as defined by:

a. 50% reduction in number of episodes of vomiting and,
b. Improvement in PO intake, as judged by the subjects and,
c. 50% reduction in the number of episodes of diarrhea as classified by the subject by the Bristol Stool Chart as type 6 or 7 bowel movements and,

2. Time from randomization to initial cessation of vomiting through Day 180 (recurrent vomiting will be considered disease recurrence unless determined to be due to another etiology [i.e. GVHD]).
3. Time from randomization to subject report of no loss of appetite that is maintained for 48 hours through Day 180 (measure will be first time that the subject reports that they have no "loss of appetite" on their daily diary).
4. Time from randomization to initial cessation of diarrhea through Day 180 classified by the Bristol Stool Chart as type 6 or 7 bowel movements (recurrent diarrhea will be considered disease recurrence unless determined to be due to another etiology [i.e. GVHD]).
5. Time from randomization to 50% reduction in the amount of antimotility agents utilized by the subject through Day 180.
6. Number of days of diarrhea through Days 28 and 180.
7. Number of stools classified by the Bristol Stool Chart as type 6 or 7 bowel movements through Day 28.
8. Number of days of IV hydration or total parenteral nutrition (TPN) therapy through Day 28.
9. Total number of days of hospitalization through Days 28 and 180.
10. Change in patient-reported quality of life as measured by EuroQOL-5 (global) and IBSQOL (diarrhea specific) [adult] or Euro QOL-5 Peds (global) and PedsQL GI Module [children] from baseline to Day 180.
11. Change in patient-reported physical function as measured by the NIH PROMIS Physical Function (Adult) Pediatric Global Health (Peds) from baseline to Day 180.
12. Change in patient-reported emotional distress as measured by the NIH PROMIS Depression, Anxiety, Fatigue (Adult and Peds) from baseline to Day 180.
13. Change in patient-reported gastrointestinal symptoms as measured by the NIH PROMIS GI (Adult and Peds) symptoms (6 of 8 subscales) from baseline to Day 180.
14. The effect of co-pathogens, as determined by the BioFire stool pathogen screen, on the primary outcome measure
15. Time from randomization until initial clinical resolution of Norovirus symptoms for at least 48 hours through Day 180. Clinical resolution will be assessed from the daily diary by the subject and will be defined as cessation of vomiting and no stools classified by the Bristol Stool Chart as diarrhea (Type 6 or 7) from the

total population compared to the subgroup of subjects who do not report chromaturia (discoloration of urine) based on unsolicited self-reporting.

16. Incidence of unsolicited non-serious adverse events (see section 9 for details) through 60 days, laboratory adverse events (WBC, Hemoglobin, Platelet count, Creatinine, Alkaline Phosphatase, ALT, AST, BUN and Bilirubin) through 60 days, protocol-specified serious adverse events (see section 9 for details) through 60 days, and Hospitalization through 60 days from the total population compared to the subgroup of subjects who do not report chromaturia (discoloration of urine) based on unsolicited self-reporting.

Virologic Improvement:

17. Time to ≥ 1 log reduction in stool Norovirus genome copies.
18. Change in quantitative Norovirus load in the stool between Day 1 and Day 7, 14, 21, 28, 60, 120, and 180.
19. Change in Norovirus sequence from Day 1 to Days 28 and 60.
20. The effect of co-pathogens, as determined by the BioFire stool pathogen screen, on the time from the randomization to first negative viral load through 180 days
21. Time from the randomization to first negative viral load through 180 days and the change in viral titer between Day 1 and 180 from the total population compared to the subgroup of subjects who do not report chromaturia (discoloration of urine) based on unsolicited self-reporting.

Immunologic Response:

22. Change in Norovirus-specific serum and stool IgA, IgM and IgG between Day 1 and Day 28 and 60 (stool only).
23. Association between change in total lymphocyte count and T cell subsets at Day 1 and 180 and clinical resolution of symptoms.
24. Association between change in total lymphocyte count and T cell subsets at Day 1 and 180 and undetectable quantitative Norovirus PCR.
25. Association between change in Norovirus-specific serum and stool IgA, IgM and IgG at from Day 1 to Days 28 and 60 (stool only) and clinical resolution of symptoms.
26. Association between change in Norovirus-specific serum and stool IgA, IgM and IgG from Day 1 and Days 28 and 60 (stool only) and undetectable quantitative Norovirus PCR.

Pharmacokinetics and Dose Response:

27. For subjects consenting to PK draws, concentrations of nitazoxanide metabolites (tizoxanide and tizoxanide glucuronide) 1 to 4 hours after the first dose on Day 7 and 10 minutes before the second dose on Day 21.

Natural History of Disease:

28. Time to allograft rejection as per each center team.
29. Time to graft loss as reported to UNOS.
30. Time to death.
31. Time to withdrawal from the study because of intolerance or drug related adverse events.
32. Correlation of secretor status as defined by phenotype and genotype, separately and clinical resolution of Norovirus as defined by the primary endpoint.

Clinical Improvement:

For exploratory outcomes 1 through 5, Kaplan-Meier curves will be produced along with results from a Log-Rank test to compare the time from randomization to clinical improvement in Norovirus disease between the nitazoxanide treated group and the placebo group. The Cox proportional hazards model will be used to estimate the corresponding hazard ratio and 95% confidence interval. If baseline demographic variables are shown to be unbalanced among the treatment groups, those variables will be adjusted in the Cox model.

For exploratory outcomes 6-8, if the number of days of diarrhea follows an approximately normal distribution, then the average number of days will be estimated and compared between the two groups. If the number of days of diarrhea does not follow a normal distribution, a Poisson distribution or non-parametric analysis such as the Wilcoxon test will be considered.

For exploratory outcomes 9, if number of days of hospitalization follows an approximately normal distribution, then the average number of days will be estimated and compared between groups using a two-sample t-test. If the number of days of hospitalization does not follow a normal distribution, a Poisson distribution or a non-parametric analysis such as the Wilcoxon test will be considered.

For exploratory outcomes 10-13, subjects will complete Detailed PRO and Symptom Severity questionnaires at each visit to track changes over time in each of the psychosocial and QOL constructs. An ANCOVA model will be fit using the two groups as the between-subjects factor, time as the within-subjects factor and baseline scores as a covariate. In addition, groups will be compared on unadjusted change over time using a mixed-design analysis of variance (ANOVA) model with all times, including baseline, as a repeated measures factor. Of interest is whether

there are statistically significant pairwise contrasts between the groups on unadjusted change between baseline and subsequent time points, and on adjusted change (controlling for baseline).

For exploratory outcome 14, a Cox proportional hazards model will be utilized to determine the effect of the presence or absence of co-pathogens at any time point on the time to clinical resolution of Norovirus symptoms for at least 48 hours through Day 180. A binary covariate representing the presence or absence of co-pathogens will be included in the model. As an alternative, if it is determined that subjects' co-pathogen status changes often throughout the study, the effect may be considered as a time-varying covariate.

For exloratoy outcome 15,16-please see section 11.4.3.3

Virologic Improvement:
For exploratory outcomes 17-19, analysis of Norovirus sequencing data, mutations will be compared between the nitazoxanide and placebo treatment groups. Non-synonymous/synonymous (dN/dS) mutation analysis will be performed for each subject to determine whether selection is occurring within the polymerase, as well as Viral Epidemiology Signature Pattern Analysis (VESPA) and Random Effects Likelihood and Fixed Effects Likelihood (REL and FEL) methods to perform site-specific analyses and to identify trends within the nitazoxanide treatment group and differences between the treatment and placebo groups.

For exploratory outcome 20, a Cox proportional hazards model will be utilized to determine the effect of the presence or absence of co-pathogens at any time point on the time to first negative viral load through 180 days. A binary covariate representing the presence or absence or co-pathogens will be included in the model. As an alternative, if it is determined that subjects' co-pathogen status changes often throughout the study, the effect may be considered as a time-varying covariate.

For exploratory outcome 21, please see section 11.4.3.3

Immunologic Response:
For stool immunoglobulin testing, Day 1 will assess baseline values, Day 28 will assess end of treatment levels and Day 60 will give a single post-treatment level. The proportion of subjects with ≥4-fold rise in Norovirus-specific Ig titers from Day 1 will be assessed at each follow-up time point comparing the nitazoxanide treatment group to the placebo group.

Serum Immunoglobulin Testing (Serum total Ig and Norovirus-specific levels): Day 1 will assess baseline values, Day 28 will assess end of treatment levels. The proportion of subjects with ≥4-fold rise in Norovirus-specific Ig titers from Day 1 will be assessed at Day 28 comparing

the nitazoxanide treatment group to the placebo group. In addition, the ratios of Norovirus-specific IgG to total IgG will also be compared between the nitazoxanide and placebo groups.

For exploratory outcome 22, an ANCOVA model will first be used to compare the treatment and placebo groups with respect to difference in changes of Norovirus-specific serum and stool IgA, IgM and IgG between Day 1 and Day 28 and between Day 1 and Day 60 (for stool only). A mixed effects model will then be used to compare the treatment and placebo groups with respect to difference in the changes of Norovirus-specific serum and stool IgA, IgM and IgG, which will use the longitudinal data observed at all three time points.

For exploratory outcomes 23-26, a Fisher's Z-transformation will be applied to the Pearson correlations between change in total lymphocyte count and T cell subsets at Day 1 and 180 and clinical resolution of symptoms in both the treatment and placebo groups, which will then be compared using a Z-test.

Pharmacokinetics and Dose Response:
For exploratory outcome 27, plasma concentrations of nitazoxanide metabolites in subjects consenting for PK blood draws at two time points will be described and summarized. Descriptive statistics (e.g. n, mean, standard deviation, % CV, median, and range) will be calculated for each sampling time.

Cox proportional hazard regression models will be used to examine the dose response relationship of Day 7 and Day 21 nitazoxanide metabolite concentrations on time from randomization until initial clinical resolution of Norovirus symptoms for at least 48 hours through Day 180. Additional analyses of PK endpoints may be described in the SAP.

Natural History of Disease:
Exploratory outcomes 28-31 are all time-to-event outcomes. They will be analyzed similarly as exploratory outcome 1.

Exploratory outcome 32 will be assessed similarly to exploratory outcome 14.

12 SOURCE DOCUMENTS AND ACCESS TO SOURCE DATA/DOCUMENTS

Each participating 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, which include, but are not limited to, hospital records, clinical and office charts, laboratory notes, memoranda, subjects' memory aid or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate and complete, microfiches, photographic negatives, microfilm or magnetic media, x-rays, and subject files and records kept at the pharmacy, at the laboratories, and medico-technical departments involved in the clinical trial.

13 QUALITY CONTROL AND QUALITY ASSURANCE

Following a written DMID-accepted protocol-specific quality management plan, each investigational site is responsible for conducting routine quality control (QC) and quality assurance (QA) activities to internally monitor study progress and protocol compliance. Each site Principal Investigator will provide direct access to all protocol-related source data/source documents, and reports for the purpose of monitoring and auditing by the sponsor, and inspection by local and regulatory authorities. Each site Principal Investigator will ensure all study personnel are appropriately trained and current documentations are maintained on site.

DMID-designated clinical monitors will verify the clinical trial data are generated, documented (recorded), and reported in compliance with the protocol, Good Clinical Practice standards, and the applicable regulatory requirements. Clinical monitoring reports will be submitted to DMID.

The Statistical and Data Coordinating Center (SDCC) will implement quality control procedures beginning with the data entry system; database quality control checks will be implemented. Any missing data or data anomalies will be communicated to the site(s) for clarification and resolution.

14 ETHICS/PROTECTION OF HUMAN SUBJECTS

14.1 Ethical Standard

The site principal investigator (PI) will ensure that this trial is conducted in full conformity with principles of the Belmont Report: Ethical Principles and Guidelines for the Protection of Human Subjects of Research (National Commission for the Protection of Human Subjects of Biomedical and Behavioral Research [April 18, 1979]) and codified in 45 CFR 46, 21 CFR 50 and 21 CFR 56, as applicable. The PI will also ensure conformity with ICH E6 Good Clinical Practice, and applicable federal regulations, guidance, and guidelines for Good Clinical Practice and Clinical Trials with humans.

14.2 Institutional Review Board

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 [*OHRP-only* or *OHRP/FDA*] 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. 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.

14.3 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 written 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. 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 a written 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 written 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.

14.3.1 Informed Consent/Assent Process (in Case of a Minor)

Investigators will follow IRB/IEC requirements for enrollment of minors in this study. Minors will be informed about the study to the extent understandable to the minor. Investigators or designee will conduct the consent process with the parent(s)/legal guardian, who will be given an IRB/IEC-approved permission form, which may be referred to as a consent form, to read, review, and sign prior to any study procedures. The parent(s)/legal guardian will be provided meaningful study information including a statement that this study involves research, the child may not benefit from the trial, and the study involves risk. The required elements will be clearly presented, including the purpose of the study, the experimental procedures, the potential risks

and discomforts, known adverse effects, possible benefits of the study for the subject, alternative therapies that may be beneficial, use and disclosure of private information, and other elements that are part of obtaining proper consent. The subject's parent(s)/legal guardian will be allowed sufficient time to discuss questions with the investigator.
The investigator or designee will describe in simplified terms the details of the study intervention/product, study procedures, risks and discomforts, benefits, and other consent elements, as appropriate. A separate IRB/IEC-approved assent form will be used for the minor, who may read and sign the form, or have it read to him/her prior to participation in study procedures. Assent may be obtained verbally or waived when approved by the IRB/IEC as appropriate to age. If a child declines to participate in the trial when assent is required by the IRB/IEC, the subject will not be enrolled even though the parents have provided permission.
To ensure that consent is an ongoing process throughout the subject's participation in the study, the investigator and staff will review information as needed with the subject and the parent(s)/legal guardian and confirm that assent and permission are continuing. The permission and assent documents will be updated when new information is acquired that may impact the decision to continue in the study, and the subject's assent and the parent(s)/legal guardian's permission will be obtained, as applicable.

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

This trial will be inclusive of all subjects who are ≥ 12 years of age and thus will follow all guidelines detailed in Subpart D – Additional Protections for Children Involved as Subjects in Research (45 CFR Part 46.401-409) who meet the Subject Inclusion/Exclusion Criteria, regardless of religion, gender, or ethnic background.

14.5 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 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.

The study monitor, representatives from the NIH/DMID, representatives from Northwestern University and their affiliates, applicable regulatory authorities, such as the FDA and the IRB, representatives of Romark, L.C., or other authorized representatives of the sponsor may inspect all documents and records required to be maintained by the investigator, including but not limited to, medical records (office, clinic, or hospital) and pharmacy records for the subjects in this study. The clinical study sites will permit access to such records.

To protect privacy, we have received a Certificate of Confidentiality. With this Certificate, the researchers cannot be forced to release information that may identify the research subject, even by a court subpoena, in any federal, state, or local civil, criminal, administrative, legislative, or other proceedings. The researchers will use the Certificate to resist any demands for information that would identify the subject, except as explained below.

The Certificate cannot be used to resist a demand for information from personnel of the United States Government that is used for auditing or evaluation of federally funded projects, like this study, or for information that must be released in order to meet the requirements of the Federal Food and Drug Administration (FDA).

A Certificate of Confidentiality does not prevent the subject from voluntarily releasing information about themselves or their involvement in this research. If any person or agency obtains a written consent to receive research information, then the researchers may not use the Certificate to withhold that information.

The Certificate of Confidentiality does not prevent the researchers from reporting without the subject's consent, information that would identify the subject as a participant in the research project regarding matters that must be legally reported including: child and elder abuse, sexual abuse, or wanting to harm themselves or others.

14.6 Study Discontinuation

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.

In the event that the study is discontinued, subjects who were randomized to study drug can continue the use of the medication at the discretion of their clinician.

14.7 Costs, Subject Compensation and Research Related Injuries There is no cost to subjects for 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 (no more than $25/visit).

If it is determined by the participating site and the site principal investigator that an injury occurred to a subject as a 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, such as giving emergency medications to stop allergic reactions to the study drug. No financial compensation will be provided to the subject by the participating site for any injury suffered due to participation in this trial

14.8 Future Use of Stored Specimens

For the duration of the study, the specimens will be stored at the Central Lab. Upon completion of the study, buccal cells and saliva samples will be destroyed and no further host genetic testing will be performed. Stool and viruses will be stored at NUCTC laboratory facilities indefinitely. IRB will review future studies, and protections of confidentiality for any future studies with the stored specimens, e.g., each sample will be encoded (labeled) only with a barcode and a unique tracking number to protect the subject's confidentiality.

Subjects may be given the option to decide if they want their samples to be used for future research or have their samples destroyed at the end of the trial. The subject's decision can be changed at any time by notifying the study doctor's in writing, and the unused identifiable samples will be destroyed. However, if the subject originally consents to future use and subsequently changes his/her decision, any data obtained prior to the withdrawal of consent may still be used for research.

All sites will follow guidelines of local IRB offices in regard to the need to obtain re-consent from minors for future use once they reach the age of consent. Samples may be shared with investigators at the other participating sites or investigators at other institutions. Samples will not be sold or used directly for production of commercial product.

15 DATA HANDLING AND RECORD KEEPING

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.

Copies of the electronic CRF (eCRF) will be provided for use as source data collection forms and maintained for recording data for each subject enrolled in the study. Data reported in the eCRF derived from source data collection forms should be consistent or the discrepancies should be explained.

DMID 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.

15.1 Data Management Responsibilities

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

Emmes will serve as the Statistical and Data Coordinating Center for this study and will be responsible for data management, quality review, analysis, and reporting of the study data.

15.2 Data Capture Methods

Clinical data (including AEs, concomitant medications data) and clinical laboratory data will be entered into a 21 CFR Part 11-compliant Internet Data Entry System (IDES) provided by the SDCC. 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 from the source documents.

15.3 Types of Data

Data for this study will include safety, laboratory (immunologic and virologic), and patient reported outcome measures-patient diary (safety, adverse events).

15.4 Timing/Reports

A final clinical study report will be prepared following availability of all safety, PK, and efficacy data. See Section 9.6.2 for additional reporting requirements.

15.5 Study Records Retention

Study documents will be retained for a minimum of 2 years after the last approval of a marketing application in an ICH region and until there are no pending or contemplated marketing applications in an ICH region or at least 2 years have elapsed since the formal discontinuation of clinical development of the investigational product. Consents with future use provisions will be retained until all samples are used or destroyed. All study documents will be retained for a longer period, however, if required by local regulations. No records will be destroyed without the written consent of the sponsor. It is the responsibility of the sponsor to inform the investigator when these documents no longer need to be retained.

15.6 Protocol Deviations

A protocol deviation is any noncompliance with the clinical study protocol, Good Clinical Practice (GCP), or Manual of Procedures requirements. The noncompliance may be either on the part of the subject, the investigator, or the study site staff. 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 to use continuous vigilance to identify and report deviations within 5 working days of identification of the protocol deviation, or within 5 working days of the scheduled protocol-required activity. All deviations must be promptly reported to DMID, via The SDDC's AdvantageEDC^{SM}.

All deviations from the protocol must be addressed in study subject source documents. A completed copy of the DMID Protocol Deviation Form must be maintained in the regulatory file, as well as in the subject's source document. Protocol deviations must be sent to the local IRB per their guidelines. The site PI/study staff is responsible for knowing and adhering to their IRB requirements.

16 PUBLICATION POLICY

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

17 LITERATURE REFERENCES

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APPENDIX A: Schedule of Events

Phase	**Treatment Phase**					**Longitudinal Monitoring Phase**								
Procedure	Screen /Enroll /Day 1	Day 7 (±3 d)	Day 14₩ (±3 d)	Day 21₩ (±3d)	Day 28 (+3 d)	Day 35 (±3 d)	Day 53 (± 7d)	Day 60₩ (±14 d)	Day 113 (± 7d)	Day 120₩ (±14 d)	Day 173 (± 7d)	Day 180 (±14 d)	Unscheduled Visit	Early Termination visit
Visit	01	02	03	04	05	06	07	08	09	10	11	12		
Consent[1]	X													
Urine Pregnancy[2]	X													
Blood Draw	22.5 mL₱	8.5 mL[3]			18.5 mL[3]							22.5 mL[3]		22.5 mL[3]
Pill Count		X			X			X[10]						
Vital Signs*	X	X			X			X				X	X	X
Medical History	X													
Physical Exam	X				X									
Inquiry about hospitalization since last Assessment		X	X	X	X	X		X		X		X		X
Collect Stool	X[8¶]	X[7¶]	X[7¶]	X[7¶]	X[87¶]			X[87¶]		X[7¶]		X[7¶]	X[7¶]	X[7¶]
Buccal Swab/Saliva Collection	X													
Randomization	X													
Nitazoxanide PK[4]		4.5 mL		4.5 mL										

Nitazoxanide or placebo[5]	X	X	X	X	X									
Return residual medication					X									X
Review concomitant medications	X	X	X	X	X	X		X		X		X		X
Assess for adverse events		X	X	X	X	X		X					X	X
Administer Detailed PROs Packet	X				X			X		X		X		X
Administer Weekly Packet	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Collect or Review Daily Diary[6]		X	X	X	X			X		X		X	X	X
Phone Call			X[a]	X[a]		X	X	X[a]	X	X[a]	X			

[1]Consent/Assent must be obtained from eligible subjects, consistent with local IRB policy for consent and assent of subjects and must be obtained prior to any study procedures being performed

[2] Urine pregnancy test will be obtained from women of childbearing potential, results of test must be known and negative prior to dosing.

[3]Values obtained within 7 days of the study visit, if obtained for clinical indication, can be utilized during the treatment phase and within 14 days of the study visit for the longitudinal monitoring phase. Blood collection includes CBC, Complete Metibolic Panel, & T Cell Subsets (T cell subset will be drawn unless CBC shows an ALC<100). Serum for Ig studies on Days 28 & 180.

[4] The PK specimen on Day 7 will be collected 1-4 hours after the subject takes the study dose and on Day 21 will be a pre-dose trough and will be drawn only in subjects consenting to this optional assessment.

[5] Nitazoxanide or placebo kit will be provided to the subject. Study drug (Nitazoxanide or placebo) given BID for 28 days.

[6] Diary recorded once a day during the treatment phase and once a day during the longitudinal monitoring phase. Copies of completed pages are collected at each study visit. One pack give an enrollment and additional copies provided at Day 28.

[7] Stool for pathogen screen will not be performed for formed stool except on Day 1 and 180. An aliquot will be retained for later testing if indicated. Norovirus testing includes quantitative viral load at all time points.

[8] Sequencing at selected time points.

[10]Day 60 pill count only needs to be conducted if the dosing was not completed and medications returned during Study Day 28.

[a] Can covert to virtual visit

[§] If diarrhea recurs after stopping therapy, would have subject provide a stool specimen for pathogen screen and Norovirus PCR; therapy is at the discretion of the site.

[¥] The sample must have been produced within 24 hours of the visit and if it exceeds 24 hours it must be disposed. After collection in the stool hat, the subjects can wipe down and disinfect the outside of the container and place it in a bag for transport then refrigerate until they come to their visit with the research staff. Subjects would be encouraged to provide another sample within the visit window and if not, rectal swab may be used for all assays related to stool collection.

*Vital Signs include weight, Blood Pressure, Respiration, Pulse, Temperature and Height. Height is only measured at Screening/Enrollment/Baseline visit.

[#]Derived from Complete Blood Count.

[€]Derived from Complete Metabolic Panel.

[¶]Specimen also stored for future use.

[₱]Must be within 3 days of enrollment. Blood collection includes CBC, Complete Metibolic Panel, T Cell Subsets, and Serum for Ig studies.

[ª]If Virtual Visit

[ʊ] If part of PK substudy

Appendix B: Laboratory Reference Ranges and Toxicity Grading

Blood, Serum, or Plasma Chemistries[1]	**Reference Range[2]**	**LO/ HI/ N[3]**	**Mild (Grade 1)**	**Moderate (Grade 2)**	**Severe (Grade 3)**
Creatinine (mg/dL)		HI	>ULN - 1.5 x ULN	>1.5 – 3.0 x ULN	>3.0 x ULN
Blood Urea Nitrogen (BUN, mg/dL)		HI	>ULN - 26	27 - 31	>31
Alkaline phosphatase (U/L)		HI	>ULN – 2.5 x ULN	>2.5 – 5.0 x ULN	>5.0 x ULN
AST (U/L)		HI	>ULN – 3.0 x ULN	>3.0 – 5.0 x ULN	>5.0 x ULN
ALT (U/L)		HI	>ULN – 3.0 x ULN	>3.0 – 5.0 x ULN	>5.0 x ULN
Bilirubin, serum total (mg/dL)		HI	>ULN – 1.5 x ULN	>1.5 – 3.0 x ULN	>3.0 x ULN

[1] Depending upon the lab used, references ranges, eligibility ranges and grading may be split out by sex and/or age.
[2] Reference range of individual study site laboratory
[3] High, Low, Not Graded
ULN – upper limit of normal; LLN – lower limit of normal

Hematology	Reference Range4	LO/HI/N5	Mild (Grade 1)6	Moderate (Grade 2)	Severe (Grade 3)
Hemoglobin (g/dL)		LO	<LLN – 10.0	<10.0 – 8.0	<8.0
White Blood Cell Count (WBC, K/CUMM)		HI	>ULN - 15.00	15.00 - 20.00	>20.00
		LO	<LLN – 3.0	<3.0 – 2.0	<2.0
Platelets (K/CUMM)		LO	<LLN - 75	<75 - 50	<50
Other Laboratory Parameters		LO /HI	Test result is associated with a mild sign or symptom.	Test result requires minimal, local or non-invasive intervention.	Test result requires an invasive medical or surgical intervention, or change in study dosing outside of the protocol defined dosing or discontinuation from the study treatment, or addition of new medication.

[4] Reference range of individual study site laboratory
[5] High, Low, Not Graded
ULN – upper limit of normal; LLN – lower limit of normal

Appendix C: Patient Diary Components

Symptom Diary	
Bristol Stool Scale	Lewis SJ, Heaton KW (1997). "Stool form scale as a useful guide to intestinal transit time". Scand. J. Gastroenterol. **32** (9): 920–4. doi:10.3109/00365529709011203. PMID 9299672. Scale is free and in public domain, no permission required
Positive and Negative Affect Scale (weekly diary only)	Public domain, http://www.parqol.com/page.cfm?id=87 Watson, D., Clark, L. A., & Tellegen, A. (1988). Development and validation of brief measures of positive and negative affect: The PANAS scales. *Journal of Psychology, 54(6),* 1063-1070.
Other individual questions about diarrhea, nausea and abdominal pain	Additional items in daily/weekly symptom diary were either validated in Dr Keefer's NIH Trial or were arrived at with expert consensus with study investigators No copyright, Dr Keefer is a Co-investigator on this trial
Detailed Patient Reported Outcome and Severity Score Assessment	
PROMIS Short Forms-- Adults	PROMIS measures in English and Spanish are publicly available without license, fee, or royalty. http://www.healthmeasures.net/explore-measurement-systems/promis; https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2829758/
Depression	
Anxiety	
Fatigue	
Sleep Disturbance	
GI symptoms	
Physical function	
Fecal incontinence	
PROMIS Short-Forms-- Pediatrics	
Depression	
Anxiety	
Fatigue	
EuroQOL-5D	Validated general QOL measure, permission obtained and study registered with company http://www.euroqol.org/
IBS-Quality of Life Scale	Validated GI specific QOL measure, Permission requested/study registered with ePROVIDE

	https://eprovide.mapi-trust.org/instruments/irritable-bowel-syndrome-quality-of-life
PedsQL4	Validated general QOL measure for pediatrics, Permission requested/study registered with PEDSQL http://www.pedsql.org/pedsql13.html