Alanyl-glutamine supplementation of standard treatment for *C. difficile* infection: A single arm trial

Protocol Number: UVAHS-144404

PI of IND/IDE: Cirle Warren, MD

IND/IDE Number: 121545

NCT 02053350

Principal Investigator: Cirle Warren, MD

Medical Monitor: Cirle Warren, MD

Draft or Version Number:

June 20, 2016

STATEMENT OF COMPLIANCE

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

- United States (US) Code of Federal Regulations (CFR) applicable to clinical studies (45 CFR Part 46; 21 CFR Part 50, 21 CFR Part 56, and 21 CFR Part 312)
- ICH E6; 62 Federal Register 25691 (1997)
- University of Virginia Institutional Review Board
- University of Virginia School of Medicine Clinical Trial Office
- University of Virginia Cancer Center Office of Clinical Research Data and Safety Monitoring Committee

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 constitutes the approval of this protocol and the attachments, and provides the necessary assurances 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 guidelines.

Principal Investigator

Signed: _____ Date: _____

Cirle Warren, MD

TABLE OF CONTENTS

LIST OF ABBREVIATIONS

AE	Adverse Event/Adverse Experience
AG	L-Alanyl-L-Glutamine
AHRQ	Agency for Healthcare Research and Quality
CDC	Centers for Disease Control and Prevention
CDI	*Clostridium difficile* infection
CFR	Code of Federal Regulations
CIOMS	Council for International Organizations of Medical Sciences
CONSORT	Consolidated Standards of Reporting Trials
CFR	Code of Federal Regulations
CRF	Case Report Form
CRO	Contract Research Organization
CTO	Clinical Trials Office
DCC	Data Coordinating Center
DHHS	Department of Health and Human Services
DSMC	Data and Safety Monitoring Committee
eCRF	Electronic Case Report Form
ELISA	Enzyme-Linked Immunosorbent Assay
EMR	Electronic Medical Record
FDA	Food and Drug Administration
FWA	Federalwide Assurance
GCP	Good Clinical Practice
HIPAA	Health Insurance Portability and Accountability Act
IB	Investigator's Brochure
ICF	Informed Consent Form
ICH	International Conference on Harmonisation
ICMJE	International Committee of Medical Journal Editors
IDE	Investigational Device Exemption
IDSA	Infectious Diseases Society of America
IEC	Independent or Institutional Ethics Committee
IND	Investigational New Drug Application
IRB	Institutional Review Board
ISM	Independent Safety Monitor
JAMA	Journal of the American Medical Association
MedDRA ®	Medical Dictionary for Regulatory Activities
MOP	Manual of Procedures
N	Number (typically refers to subjects)
NDA	New Drug Application
NEJM	New England Journal of Medicine
NIH	National Institutes of Health
OHRP	Office for Human Research Protections
PCR	Polymerase Chain Reaction

PHI	Protected Health Information
PI	Principal Investigator
PK	Pharmacokinetics
QA	Quality Assurance
QC	Quality Control
SAE	Serious Adverse Event/Serious Adverse Experience
SHEA	The Society for Healthcare Epidemiology of America
SMC	Safety Monitoring Committee
SOP	Standard Operating Procedure
TPN	Total Parenteral Nutrition
US	United States
UVA	University of Virginia
WHO	World Health Organization

PROTOCOL SUMMARY

Limit to 1-2 pages

Title:	**Alanyl-glutamine supplementation of standard treatment for *C. difficile infection*: A single arm trial**
Phase:	II
Population:	The study population will include 43 participants aged ≥ 18 years,hospitalized patients or UVA outpatients. Both genders will be included. Participants must have mild to moderate or severe uncomplicated *C. difficile* infection, and be able to participate in follow-up. The trial will be held in Central Virginia.
Number of Sites:	One site – University of Virginia Health System
Study Duration:	The study duration is four years.
Subject Participation Duration:	Each individual participant will be followed for a total of 6 months post-treatment.
Description of Agent or Intervention:	All participants will receive 44 grams of alanyl-glutamine dipeptide . The supplement will be in a powder form that will be mixed with any juice or fluid and taken by mouth or enteral feeding tube.
Objectives:	Primary Outcome: Clinical failure (death and/or CDI recurrence assessed at D40 and/or lack of clinical cure as described in Section 3.2.1) – Participants will be followed up by telephone (or in person if still hospitalized) at days 40, 70, 190 post-enrollment. They will be asked about recurrences of diarrhea and diagnoses of CDI in between follow up calls. The electronic medical record (EMR) of the UVa Health System will be reviewed during these telephone or follow up visits to identify clinic or hospital encounters for diarrhea or CDI that may have occurred in the interim. Secondary Outcomes : CDI recurrence at days 70 and 190 Duration of diarrhea – Duration of diarrhea will be determined by

	daily visits or phone calls (if hospitalized participant is discharged) while participant is on the study agent (D1 to D10). Mortality at days 70 and 190– Mortality (CDI and all-cause) will be determined during the follow-up phone calls and by reviewing the EMR.
Description of Study Design:	See Schematic of Study Design below.
Estimated Time to Complete Enrollment:	100% of participants will be enrolled at 48 months.

Schematic of Study Design:

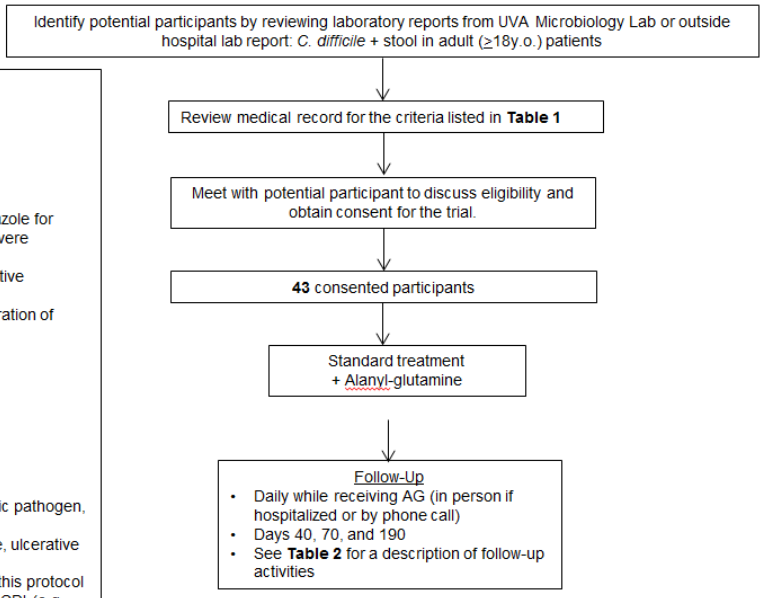


Table 1.

Inclusion Criteria

- Either gender
- Aged ≥ 18 years of age
- Hospitalized or outpatients
- *C. difficile* positive stool
- Diarrhea at time of stool testing
- Within 72 hours of receiving either oral metronidazole for mild-moderate disease or oral vancomycin for severe uncomplicated disease
- Ability to provide informed consent or if with cognitive impairment, has a surrogate
- Ability to comply with study procedures for the duration of study agent administration

Exclusion Criteria

- Hypotension or shock
- Megacolon or moderate to severe ileus
- Acute abdomen
- Severe leukocytosis (WBC ≥ 25,000 /µL)
- Admission to intensive care unit on enrollment
- Inability to tolerate oral or enteral medication
- Other known etiology of diarrhea (e.g. other enteric pathogen, other intestinal disease)
- Inflammatory bowel disease (e.g. Crohn's disease, ulcerative colitis)
- Enrollment in another trial that may interfere with this protocol
- Currently receiving other alternative treatment for CDI (e.g. antibiotics other than oral metronidazole or oral vancomycin; probiotics; IVIg; fecal transplant)
- Cirrhosis or ALT ≥ 3X normal
- End stage renal disease or in participants with or without known kidney disease, creatinine clearance or estimated GFR of ≤30mL/min even after adequate hydration
- Pregnancy
- Absolute neutrophil count of < 500/ µL
- History of organ or hematologic transplantation within the past year
- Unavailable for follow up visits

Table 2. Follow-Up

Visit Activities	Days Post-enrollment							
	0	1-4	5	6-9	10± 2	40± 7	70± 7	190 ± 7
Screening/Consenting/Enrollment	X							
Alanyl-glutamine treatment		X	X	X	X			
Clinical monitoring and follow-up	X	X	X	X	X	X	X	X
Blood, stool collection	X							

1 KEY ROLES

Principal Investigator: Cirle Warren, MD

Medical Monitor: Cirle Warren, MD

Institutions: University of Virginia Health System
Address: Division of Infectious Diseases and International Health, 345 Crispell Drive, Charlottesville, VA 22908
Phone Number: 434-924-9676
Fax Number: 434-924-0075
Email: ca6t@virginia.edu

Co-investigators:

Jae Shin, M.D.
University of Virginia Health System
Address: Division of Infectious Diseases and International Health, 345 Crispell Drive, Charlottesville, VA 22908
Phone Number: 434-924-9672
Fax Number: 434-924-0075
Email: jhs9ff@virginia.edu

Clinical Pharmacist: Amy Adams, PharmD
Address: Pharmacy Services, P. O. Box 800674, Charlottesville, VA 22908
Phone Number: 434-982-1048
Fax Number: 434-982-2900
Email: apa4n@hscmail.mcc.virginia.edu

Statistician: Gina Petroni, PhD
Address: Public Health Sciences, P. O. Box 800717, Charlottesville, VA 22908
Phone Number: 434-924-8363
Fax Number: 434-243-5787
Email: grp4c@virginia.edu

2 BACKGROUND INFORMATION AND SCIENTIFIC RATIONALE

2.1 Background Information

2.1.1 Description of the Study Agent(s)/Intervention(s)

AG is a dipeptide with a glutamine amino group joined to an alanyl residue. It has the following chemical structure: $C_8H_{15}N_3O_4$. It is a non-animal product available in the form of white crystals or crystalline powder. Ziegler et al. performed a series of dose response studies to study clinical safety, pharmacokinetics, and metabolic effects of glutamine given to humans. Participants in the studies included healthy individuals as well as bone marrow transplant patients. Glutamine was given intravenously as well as enterally at different doses. No adverse events were detected.[1] Another study which evaluated post-operative patients receiving TPN supplemented with AG did not reveal any adverse effects compared to controls which received TPN without AG.[2] These 2 studies were included in a review of 4 clinical trials specifically looking at safety of glutamine by Peter Garlick et al (2001 in *The Journal of Nutrition)* who concluded that no adverse effects of glutamine have been demonstrated in doses of up to 50-60 grams/day. While the long-term effects of chronic supplementation (≥ 30 days) are unknown, no adverse events have been demonstrated in these short-term studies.[3]

The chemical properties of free glutamine limit its use in clinical trials. Problems include instability during sterilization and storage as well as limited solubility. The breakdown products of free glutamine include glutamate and ammonia. AG is a stable, highly soluble dipeptide.[4] In a study from the United Kingdom, glutamine absorption was studied in men given different formulations of glutamine – L-glutamine, AG, and a hydrolyzed wheat protein containing L-glutamine. Higher plasma levels of glutamine were measured in the men given AG.[5]

2.1.2 Summary of Previous Pre-clinical Studies

Glutamine is an amino acid that serves as an important energy source in the body, particularly for enterocytes. It is a non-essential amino acid in healthy people but is considered "conditionally essential" during critical illness, injury, and other stressful states.[6] Utilization is increased during these times. In cellular studies involving small intestinal cell lines, glutamine was found to stimulate cell proliferation and inhibit apoptosis.[7-9] Animal models have shown that glutamine and glutamine dipeptide (e. g, AG) enhance gut repair mechanisms,[10,11] decrease enteritis/colitis,[12,13] and reduce bacterial translocation.[14,15] Glutamine-enriched tube feeding has been shown to limit weight loss in rats after small bowel resection. This study also demonstrated an increase in ileal hyperplasia in the rats receiving a glutamine-enriched diet, suggesting that glutamine may improve intestinal adaption after surgical resection.[16]

In our laboratory, the cellular effects of *C. difficile* toxins as well as AG supplementation have been studied extensively. The toxins exert their effects by inhibiting Rho proteins involved in maintaining cytoskeletal structure and by activating caspase enzymes involved in apoptosis. Rat intestinal cells treated with *C. difficile* toxin A and AG demonstrate an increase in Rho expression as well as a reduction in apoptosis and cellular damage.[17,18] In studies using rabbit and murine cecal loops exposed to toxin A, AG supplementation results in decreased ileal secretion and epithelial injury.[19] AG decreases apoptosis and inhibits activation of caspase 8 in human intestinal epithelial cells.[20] In animal studies, AG has been shown to have beneficial effects in CDI. In a murine model of CDI, animals who received vancomycin initially had improved clinical scores and survival during treatment. However, after vancomycin was discontinued, 62% of mice experienced CDI recurrence and mortality rates were similar to the untreated control group.[21] Vancomycin treatment supplemented with AG resulted in milder symptoms and improved mortality. Histopathologic analysis revealed less inflammation and fewer apoptotic cells.[22]

2.1.3 Summary of Relevant Clinical Studies

Numerous clinical trials have evaluated the effects of glutamine. In a randomized, double-blind pilot trial at the University of Colorado, researchers assessed the safety and feasibility of oral glutamine administration. Patients undergoing elective cardiac surgery (requiring cardiopulmonary bypass) were given oral AG or maltodextrin daily for 3 days pre-operatively. Serum troponin, creatine kinase, and myoglobin levels were measured during the perioperative period and clinical outcomes were recorded. The patients receiving AG had lower levels of these serum markers as well as fewer clinical complications. The authors demonstrated that this therapy is safe and feasible. A larger clinical trial is needed to evaluate the clinical efficacy or glutamine supplementation prior to cardiac surgery.[23]

In a randomized, double-blind, placebo controlled trial from northern Brazil, 10 days of oral AG supplementation improved intestinal absorption in a group of HIV-positive patients. This was demonstrated by an increase in urinary mannitol excretion.[24] The same researchers performed another randomized, double-blind placebo controlled study to evaluate the effects of oral AG supplementation on absorption of anti-retroviral agents. The study showed that HIV-positive patients receiving glutamine experienced less diarrhea compared to the controls who received glycine. Higher serum levels of anti-retroviral drugs were measured in the patients who received glutamine.[25]

In a pilot study at Indiana University, HIV-positive men with diarrhea were treated with soluble fiber and probiotics. Glutamine was added to the regimen if their diarrheal symptoms did not resolve. The men who received glutamine supplementation experienced an improvement in their diarrhea.[26] Of note, the company which supplied the glutamine for the study, Ajinomoto™, will also supply the AG for our study.

In cancer patients, glutamine has been evaluated as a potential supplement in managing the dose-limiting side effects of chemotherapy. It has been associated with a reduction in irinotecan-related diarrhea in patients with colon cancer.[27] In a non-randomized study of

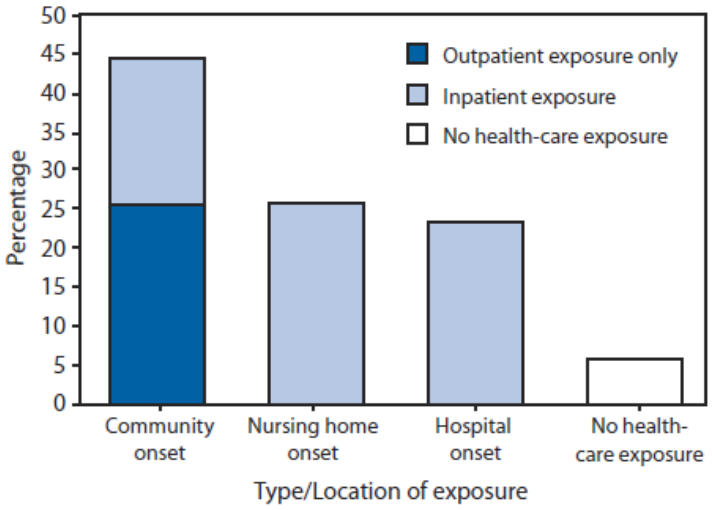
patients receiving paclitaxel, the participants given glutamine experienced less weakness and sensory loss compared to controls.[28] In another group of patients receiving oxaliplatin, less neurotoxicity was observed.[29]

2.1.4 Summary of Epidemiological Data

While CDI is classically associated with antibiotic exposure, an increasing number of cases have been seen in individuals who have not taken antibiotics, but have been exposed to the healthcare system. The increase in incidence and mortality has been attributed to the emergence of the BI/NAP/027 strain. Government agencies including the Centers for Disease Control and Prevention (CDC) and the Agency for Healthcare Research and Quality (AHRQ) are closely monitoring this infection. The Healthcare Cost and Utilization Project (HCUP), sponsored by the AHRQ, has published data on the rates of CDI. HCUP consists of a group of databases which contain information related to diagnoses, procedures, discharge status, and patient demographics. Per data from HCUP, there were 336,000 hospitalizations which involved CDI in 2009. This is an increase from 139,000 hospitalizations in 2000. CDI as the principal diagnosis increased from 32,800 hospital stays to 110,600 during the same time period.[30] Patients hospitalized with CDI are typically more ill than other hospitalized patients. In addition to diarrhea, the infection is associated with dehydration, electrolyte imbalances, sepsis/septic shock, renal failure, intestinal perforation, and toxic megacolon.

Data collected the by CDC's Emerging Infections Program indicates that 94% of CDI was associated with some type of healthcare exposure (Figure 1). 25% of patients have symptoms while hospitalized whereas the remaining 75% develop symptoms in a nursing home or after receiving care at a clinic.[31] When this latter group of patients presents at an acute care hospital, they are considered to have CDI that is 'present on admission.' Depending upon the diagnostic test used at the facility (i.e. toxin assay or PCR), the diagnosis may not be confirmed for 24-48 hours. During that period, the patients may not be placed on appropriate isolation precautions and the risk for transmission to other patients by healthcare workers is increased. CDI that is 'present on admission' serves as an important source of intra-hospital transmission and is a risk factor for higher hospital-acquired CDI rates.[31] The incubation period for CDI is approximately 2-3 days so patients are more likely to have acquired CDI in the setting in which symptom began.

FIGURE 1. Percentage of *Clostridium difficile* infection (CDI) cases (N = 10,342), by inpatient or outpatient status at time of stool collection and type/location of exposures* — United States, Emerging Infections Program, 2010



Source: CDC[31]

2.2 Rationale

Hypothesis: Supplementation of standard CDI treatment with AG will decrease recurrence, improve symptoms, and reduce mortality associated with the disease.

Participants will be given AG 44 grams by mouth (or by enteral feeding tube) daily for 10 days. AG will be prepared in powder form, mixed with any juice or fluid, and taken orally (or enterally). These supplements will be given in addition to standard antibiotic treatment for CDI. The dose was chosen based on a prior study.[25] Daily dosing was chosen for ease of administration in participants who are no longer hospitalized – i.e. the participant will be responsible for taking the drug rather than administration by nursing staff. The duration of intervention is 10 days, regardless of the duration of antibiotic treatment for CDI.

Our study population includes all adult inpatients, including those diagnosed with cancer – currently diagnosed or a prior history as well as outpatients seen in UVA clinics. These individuals have many exposures to the healthcare system, a known risk factor for acquiring *C. difficile*. This particular population with malignancy is also at an increased risk for the infection due to being immunocompromised from chemotherapy, radiation, or surgical procedures.

2.3 Potential Risks and Benefits

2.3.1 Potential Risks

In a study published in the New England Journal of Medicine, critically ill patients with multiorgan failure were given glutamine supplementation (intravenously and enterally) or placebo. At 28 days, a trend toward increased mortality was noticed in the glutamine group compared to controls (32.4% vs 27.2%; adjusted odds ratio 1.28; 95% confidence interval, 1.00-1.64, P=0.05). In-hospital mortality and mortality at 6 months were higher among patients given glutamine.[32] Serious adverse events recorded during this study include: nervous system disorders (encephalopathy, intracranial hemorrhage, seizures, stroke), respiratory disorders (respiratory distress), cardiac disorders (acute cardiac failure, cardiac arrest, cardio-respiratory arrest, pericarditis, arrhythmia), gastrointestinal diseases (acute pancreatitis, gastrointestinal hemorrhage, ileus, ischemic bowel), hepatobiliary disorders (cholestasis), vascular disorders (peripheral vascular disease), eye disorders (blindness), and procedural complications (embolism at central line, tube malposition). There were no differences among the groups with respect to serious adverse events (P=0.83).[32]

Another study of 7 patients identified liver toxicity as a potential risk associated with glutamine supplementation. However, these patients were receiving TPN. The authors hypothesized that the elevation in transaminases was possibly a complication of intravenous nutrition. No control group was used in the study.[33] Patients with liver disease may have decreased ammonia excretion and may be predisposed to the accumulation of ammonia, a breakdown product of glutamine. Similarly, patients with kidney disease may have decreased renal excretion of ammonia and glutamic acid.[34]

Of note, in this proposal, patients who are critically-ill are excluded. Alanyl-glutamine will be given orally or enterally only and limited to 10 days of administration. Per review of clinical trials specifically addressing safety, glutamine at a dose of up to 60 g/day given as short as 4 hours to up to 30 days, administered to either healthy volunteers, bone marrow transplant patients, or preterm neonates, was considered safe and not associated with adverse events[3].

2.3.2 Known Potential Benefits

Based upon prior research involving cellular and animal models, potential benefits include decreased *C.difficile* toxin-mediated effects subsequently leading to less infection-induced inflammatory damage and increased epithelial repair. Participants may experience decreased diarrhea, reduced recurrence of CDI, and improved survival. These latter benefits may lead to an improved quality of life and fewer hospitalizations.

3 OBJECTIVES

3.1 Study Objectives

We propose to perform a single arm trial to estimate the effect of AG supplementation in participants receiving antibiotic treatment for CDI. The dosing regimen for AG is as follows: 44 grams of AG given by mouth (or by enteral feeding tube) daily for ten days. The primary objective is to estimate the effect of AG on day 40 clinical failure. Secondary objectives include estimation of CDI recurrence at days 70 and 190, duration of diarrhea, and CDI-associated and all-cause mortality. The exploratory objectives (only for inpatient participants) include determining the extent of (1) intestinal inflammation (fecal lactoferrin), (2) intestinal tissue integrity, (3) systemic inflammation, and (4) intestinal infection.

3.2 Study Outcome Measures

3.2.1 Primary Outcome Measures

Day 40 clinical failure – clinical failure includes death or CDI recurrence assessed 40 days post initiation of study agent or lack of clinical cure as described below.

Death – death from any cause.

CDI recurrence –CDI recurrence is determined by documentation of diarrhea and stool positive for *C. difficile* toxin.

Clinical cure – resolution of diarrhea (i.e., 3 or fewer unformed stools for 2 consecutive days), with maintenance of resolution for the duration of therapy or marked reduction in the number of unformed stools at the end of treatment and no further requirement (in the investigator's opinion) for therapy for CDI as of the second day after the end of the course of therapy (36).

3.2.2 Secondary Outcome Measures

CDI recurrence at days 70 and 190

Duration of diarrhea – Duration of diarrhea will be determined using a symptom diary of gastrointestinal symptoms. Mortality – Mortality (CDI and all-cause) will be determined during the follow-up phone calls and by reviewing the EMR.

Mortality – Mortality (CDI and all-cause) will be determined during the follow-up phone calls and by reviewing the EMR.

4 STUDY DESIGN

We propose to perform a single arm trial to estimate the effect of AG supplementation on the resolution of diarrhea during treatment, on recurrence of CDI after treatment, and on mortality at Days 40±7, 70±7 and 190±7 post-enrollment. The study will include hospitalized patients or outpatients seen in UVA clinics.

4.1 Study Endpoints

4.1.1 Primary Endpoint

CDI recurrence – The primary outcome will be measured by monitoring length of time to CDI recurrence post-enrollment. CDI recurrence is determined by documentation of diarrhea and stool positive for *C. difficile* toxin. Participants will be followed up by telephone (or in person if still hospitalized) at Days 40, 70 and 190 post-enrollment. During these "visits" or telephone calls, the participant and investigator will refer to the symptom diary for recurrence of diarrhea and associated symptoms, clinic/doctor visit or not, stool testing or any interventions that may have occurred since the last encounter. The EMR will be reviewed during these telephone or follow up visits to identify clinic or hospital encounters for diarrhea or CDI that may have occurred in the interim. For consultation and stool testing done outside of the UVA Health System, we shall obtain documentation of the clinic/hospital visits and test results.

4.1.2 Secondary Endpoints

Duration of diarrhea – Duration of diarrhea associated with the initial CDI diagnosis will be determined using a symptom diary of gastrointestinal symptoms. This diary will be given to the participants at enrollment. If the participant is discharged during the treatment period (days 1 to 10), the participant will be called daily to ask for occurrence of diarrhea and other symptoms such as abdominal pain, nausea and vomiting, and others. Mortality – Mortality (CDI and all-cause) will be determined during the follow-up phone calls and by reviewing the EMR.

5 STUDY ENROLLMENT AND WITHDRAWAL

5.1 Participant Inclusion Criteria

Participants must meet all of the inclusion criteria in order to be eligible to participate in the study.

Inclusion Criteria

- Adult of either gender, 18 years or older
- *C. difficile* positive stool
- Diarrhea at time of stool testing
- Within 72 hours of receiving either oral metronidazole for mild-moderate disease or oral vancomycin for severe uncomplicated disease
- Admitted in the hospital or outpatient at the time of enrollment
- Ability to provide informed consent or if with cognitive impairment, has a surrogate
- Have an understanding of study procedures
- Ability to comply with study procedures for the duration of study agent administration

Contraceptive methods – Given the current rates of CDI at UVA Health System, we expect that most participants will be beyond their reproductive years (mean age 61 years). Female participants will be excluded from the trial if they become pregnant. Based upon current studies, we do not expect any adverse events to the fetus. Female participants can use whichever method of contraception that they prefer.

5.2 Participant Exclusion Criteria

Participants that meet any of the exclusion criteria at baseline will be excluded from study participation.

Exclusion Criteria

- Hypotension or shock
- Megacolon or moderate to severe ileus
- Acute abdomen
- Severe leukocytosis (WBC $\geq$ 25,000 cells /μL)
- Admission to intensive care unit on enrollment

- Inability to tolerate oral or enteral medication
- Other known etiology of diarrhea (e.g. other enteric pathogen, other intestinal disease)
- Inflammatory bowel disease (e.g. Crohn's disease, ulcerative colitis)
- Enrollment in another clinical trial that may interfere with this protocol
- Receiving other alternative treatment for CDI during course of study agent administration (e.g. antibiotics other than oral metronidazole or oral vancomycin; probiotics; immunoglobulin therapy; fecal transplant)
- Cirrhosis or ALT $\geq$ 3x normal.
- End stage renal disease or in participants with or without known kidney disease, estimated Creatinine clearance or glomerular filtration rate of $\leq$ 30 ml/min, even after adequate rehydration.
- Pregnancy
- Unavailable for follow up visits
- Absolute neutrophil count of < 500/μL
- History of organ or hematologic transplantation within the past year
-

5.3 Strategies for Recruitment and Retention

Patients will be recruited if they meet the criteria listed above upon review of the medical record. While receiving the treatment, the study team will follow the participant daily if hospitalized. Outpatients or discharged inpatients will be followed-up by phone calls (or in person if still hospitalized) daily if discharged before day 10 and at Days 40, 70 and 190 post-enrollment.

5.4 Treatment Assignment Procedures

Participants who meet all eligibility criteria and consent to participation will be stratified according to disease severity (mild-moderate or severe disease) and recurrent CDI (yes or no). Mild-moderate disease is defined as a leukocyte count of ≤ 15,000 cells/μL and a serum creatinine <1.5 times the premorbid level. Severe disease is defined as a leukocyte count of > 15,000 cells/μL or a serum creatinine level ≥ 1.5 times the premorbid level.[35]

5.4.1 Randomization Procedures

There is no randomization for this trial. All participants enrolled in the study will receive the study agents described in Section 11.3.

5.4.2 Masking Procedures

None.

5.4.3 Reasons for Withdrawal

A study participant will be discontinued from protocol treatment if any of the following occurs:

- Any clinical adverse event (AE), laboratory abnormality, intercurrent illness, or other medical condition or situation occurs such that continued protocol treatment would not be in the best interest of the subject.
- Development of any exclusion criteria

Participants are free to withdraw from participating in the study at any time. Participants may also withdraw voluntarily from receiving the study intervention for any reason.

5.4.4 Handling of Withdrawals

For participants who voluntarily withdraw from the study, we will continue to follow them (if they agree) in the same manner as trial participants – daily follow-up for the 10 days after starting the treatment and telephone follow-up at Days 40, 70 and 190 (using the same questions on the symptom diary) post-enrollment.

5.4.5 Termination of Study

Reasons for termination of the study treatment include development of laboratory toxicities not attributable to other causes, study closure as recommended by the UVA IRB or Cancer Center DSMC, and increased number of SAEs as described in Section 9.2.3.

5.5 Participant Compensation

Participants will be given $50 to compensate for time spent during follow-up visits. $25 will be mailed upon completion of Day 10 follow-up and another $25 will be mailed following Day 190 follow-up.

6 STUDY INTERVENTION/INVESTIGATIONAL PRODUCT

6.1 Study Product Description

6.1.1 Acquisition

We will purchase the Study Agent- AG, from Ajinomoto™.

6.1.2 Formulation, Packaging, and Labeling

AG will be in white crystalline formulation and will be appropriately labeled as L-alanyl-L-glutamine in 20 kgs packages when received from Ajinomoto. This stock agent will be directly shipped to the UVA Pharmacy Services. Upon receipt of the study agent, the Pharmacy will repackage the agent into 44 g (AG) or doses placed in coded containers.

6.1.3 Product Storage and Stability

The product is shipped at ambient temperature and humidity. It must be stored in a dry place at ambient temperatures away from strong odors. The retest date is 48 months from the date of manufacture.

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

The Pharmacy will prepare the 44g packets of AG in containers and coded appropriately. Once in containers , agents will be discarded if not used within 6 months. The agents will be dissolved in 250ml of any juice or fluid, and will be administered by mouth or enteral tube daily for 10 days. There will be no make up for missed doses.

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

The study agent will be directly shipped, repackaged, stored in our pharmacy (drug repository) and distributed on as needed basis. The pharmacy will dispense the study agent in containers, daily while participants are in the hospital and will prepare the containers with study agent to be sent home for participants discharged before day 10 post-enrollment or for outpatient participants Any unused products will be transferred to our research laboratory at the conclusion of the study.

6.4 Assessment of Participant Compliance with Study Intervention/Investigational Product

While participants are hospitalized, the ward nurse-in-charge of the participant for that day will directly observe administration of the study agents. A clinical pharmacist will also follow-up daily with the participant in the hospital while taking the study agent per standard of care. A member of the study team will visit the participant daily to confirm intake of the study agent from the nurse and documentation in EPIC and record intake of study agent daily in the CRF. For outpatients and discharged inpatients,if the participant is discharged before completion of treatment a member of the study team will call the participant daily, to ask if study agent was taken, throughout the rest of the 10 day treatment period.

6.5 Concomitant Medications/Treatments

6.5.1 Prohibited Medications and Procedures

Prohibited medications/procedures include investigational drugs in other trials, anti-*C. difficile* antibiotics other than oral metronidazole and oral vancomycin, probiotics, immunoglobulin therapy, and fecal transplant. Patients who are on prohibited medications and procedures are excluded from the study.

6.5.2 Precautionary Medications and Procedures

We do not expect any drug-study agent interaction. Any medications or procedures not mentioned under the exclusion criteria are allowed.

7 STUDY SCHEDULE

7.1 Screening

Study researchers will review clinical microbiology laboratory reports in hospital's Microbiology laboratory daily to identify patients whose stool tested positive for *C. difficile*. Both genders will be eligible for the study. The EMR will be reviewed to determine if the patient meets the inclusion and exclusion criteria listed in Sections 5.1 and 5.2. If the patient meets eligibility criteria, the team member will meet with the patient to discuss the trial including the purpose of the study, the study intervention and other study procedures including follow-up visits, specimen collection, time commitment, and compensation. Signed informed consent will be obtained from all participants. Participants will be consented as long as the study agent can be administered within 72 hours of initiation of oral metronidazole or oral vancomycin.

7.2 Enrollment/Baseline

The EMR will be used to assess whether or not the patient meets eligibility criteria and may be enrolled. The criteria are listed in Sections 5.1 and 5.2. At baseline, the participant will be asked questions regarding his current gastrointestinal symptoms. The supplement will be taken by mouth or by enteral tube, if applicable. It is unlikely that the participant will experience any adverse reactions to the intervention so vital signs will be monitored as ordered by the treating physician during the admission process.

7.3 Follow-up

Please refer to the Schedule of Events in Appendix A for a list of the follow-up visits. The Schedule of Events also includes which labs will be drawn at each visit. For outpatients, or inpatients, if discharged during the treatment period (days 1 to 10), the participant will be called daily to ask for occurrence of diarrhea and other symptoms such as abdominal pain, nausea and vomiting, and other gastrointestinal symptoms. The participant and the investigator will refer to the symptom diary for Days 40, 70 and 190-follow up "visits" or telephone calls, for recurrence of diarrhea and associated symptoms, clinic/doctor visit or not, stool testing or any interventions that occurred. The EMR will be reviewed during the visits to identify clinic or hospital encounters for diarrhea or CDI that may have occurred in the interim. We will also assess AEs at the follow-up visits during the treatment period. Allowable windows for follow-up visits will include $\pm$2 days for Day 10 follow-up/specimen collection and $\pm$1 week for Days 40, 70 and 190 follow-up visits.

7.4 Final Study Visit

The final study visit (via telephone call) will occur at day 190 post-enrollment. The participant will be asked about recurrence of diarrhea or CDI diagnoses as described above (Section 7.3). The EMR will be reviewed to identify clinic or hospital encounters for diarrhea or CDI that may have occurred in the interim.

7.5 Early Termination Visit

There are no specific evaluations which should be done at a termination visit.

7.6 Unscheduled Visit

Participants will be evaluated at the Infectious Diseases Clinic at UVA.

7.7 Pregnancy Visit

As mentioned previously, given the mean age of participants with CDI, pregnancy is unlikely to be a common event. Participants who become pregnant will be excluded from the trial and will continue routine prenatal care with their obstetrician.

8 STUDY PROCEDURES/EVALUATIONS

8.1 Clinical Evaluations

Medical History – The EMR will be reviewed as part of the enrollment process to determine if the patient meets the inclusion and exclusion criteria (listed in Sections 5.1 and 5.2). At each follow up visit, the participant will be interviewed (via telephone or in person) about the presence of any gastrointestinal symptoms or CDI diagnoses. The EMR will also be reviewed during the follow-up visits to identify any clinic or hospital encounters for diarrhea or CDI that may have occurred in the interim.

Medications history – During enrollment, the participant will be asked about currently taken medications including prescription and over-the-counter medications. At each follow-up visit, the participant will be asked about new medications (prescription and over-the-counter).

Physical examination – The participant's vital signs will be reviewed – blood pressure, heart rate, respiratory rate, and temperature.

Review of symptom diary – The participant will be provided with a symptom diary during enrollment. The symptom diary will be reviewed at each follow-up visit.

8.2 Laboratory Evaluations

8.2.1 Clinical Laboratory Evaluations

University of Virginia Clinical Laboratory

Hematology: White blood cell count, Red blood cell count, Hemoglobin, Hematocrit, platelet count

Biochemistry: Sodium, potassium, chloride, bicarbonate, BUN, creatinine, glucose, albumin, total bilirubin, alkaline phosphatase, AST, ALT erythrocyte sedimentation rate, C-reactive protein, and if needed, serum pregnancy test (HCG qualitative)

University of Virginia Clinical Microbiology Laboratory

Stool: *C. difficile* toxin B PCR

Blood volume: 5-10ml at baseline will be collected if there are no leftover sera for add-on laboratory tests for screening purposes. Stool volume: Leftover stool specimens from baseline C.difficile positive sample will be collected from the Clinical laboratory.

**In order to exclude pregnant patients, female participants <50 years of age will be questioned regarding prior surgeries including tubal ligation and hysterectomy. The EMR will also be reviewed. For those who may be pregnant, a serum pregnancy test will be completed within 7 days prior to study agent administration. Results must be available prior to administration of the study product.

8.2.2 Special Assays or Procedures

8.2.3 Specimen Preparation, Handling, and Shipping

8.2.3.1 Instructions for Specimen Preparation, Handling, and Storage

Stool – On Day 0, any remaining stool will be collected from the Clinical Microbiology Laboratory within 24 hours of collection date. There will be no additional stool collection if specimens are not available from the clinical laboratory. It will be brought to the Warren Lab, de-identified, coded, and stored in the -80°F freezer until needed for future studies. Future studies include lactoferrin assay, *C. difficile* toxin B PCR, *C. difficile* toxin A and B ELISA, *C. difficile* culture and ribotyping..

8.2.3.2 Specimen Shipment

9 ASSESSMENT OF SAFETY

9.1 Specification of Safety Parameters

CDI recurrence – CDI recurrence will be assessed at Days 40, 70 and 190 post-enrollment. CDI recurrence is determined by documentation of diarrhea and stool positive for *C. difficile* toxin. Participants will be followed up by telephone (or in person if still hospitalized) at Days 40, 70 and 190 post-enrollment. They will be asked about recurrences of diarrhea and diagnoses of CDI in between follow up calls. The EMR will be reviewed during these telephone or follow up visits to identify clinic or hospital encounters for diarrhea or CDI that may have occurred in the interim.

Duration of diarrhea – Duration of diarrhea will be assessed daily while participant is on the study agent. This will be performed by actual visits while participant is in the hospital or by phone calls if the participant has been discharged prior to Day 10 or is an outpatient participant.

Mortality – Mortality (CDI and all-cause) will be determined during the follow-up phone calls and by reviewing the EMR.

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

9.2.1 Adverse Events

Adverse Event: ICH E6 defines an AE as any untoward medical occurrence in a clinical investigation participant administered a pharmaceutical product regardless of its causal relationship to the study treatment. 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.

All AEs including local and systemic reactions not meeting the criteria for “serious adverse events” should be captured on the appropriate CRF. Information to be collected 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, which would include MD, PA, Nurse Practitioner, DO, or DDS), and time of resolution/stabilization of the event. All AEs occurring while on study must be documented appropriately regardless of relationship. All AEs should be followed to adequate resolution.

Any medical condition that is present at the time that the participant 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.

The adverse event collection interval for this study is from the start of treatment until a minimum of 7 days following last dose of study treatment. SAEs considered related to study drug may be reported until the 190 day follow-up.

All AEs must be graded for severity, seriousness and relationship to study product, as defined below.

Severity of Event: The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 will be utilized for AE reporting. All appropriate treatment areas should have access to a copy of the CTCAE version 4.0. A copy of the CTCAE version 4.0 can be downloaded from the CTEP web site (http://ctep.cancer.gov).

To assess severity of adverse events not included in the CTCAE version 4.0, use Table below.

Table. Adverse Event Severity Grading Scale for Adverse Events Not Specifically Listed in the NCI CTCAE

Grade	Severity
1	Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.
2	Moderate; minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental ADL[a]
3	Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self care ADL[b]
4	Life-threatening consequences; urgent intervention indicated.
5	Death related to AE

a. Instrumental ADL refer to preparing meals, shopping for groceries or clothes, using the telephone, managing money, etc.
b. Self care ADL refer to bathing, dressing and undressing, feeding self, using the toilet, taking medications, and not bedridden.

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

Relationship to Study Products: The clinician's assessment of an AE's relationship to test article (vaccine or 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. All AEs must have their relationship to study product assessed using the terms: associated or not associated. In a clinical trial, the study product must always be suspect. To help assess, the following guidelines are used.

- Associated – The event is temporally related to the administration of the study product and no other etiology explains the event. For the purposes of reporting to the DSMC, this is consistent with the definitions of possible, probable and definite.

- Not Associated – The event is temporally independent of study product and/or the event appears to be explained by another etiology. For the purposes of reporting to the DSMC, this is consistent with the definitions of unrelated and unlikely.

9.2.2 Serious Adverse Events

Serious Adverse Event (SAE): An SAE is defined as an AE that meets one of the following conditions:

- Death during the period of protocol defined surveillance
- Life-threatening event (defined as a participant at immediate risk of death at the time of the event)
- An event requiring inpatient hospitalization for more than 24 hours or prolongation of existing hospitalization during the period of protocol defined surveillance*
- Results in congenital anomaly or birth defect
- Results in a persistent or significant disability/incapacity
- Any other important medical event that may not result in death, be life threatening, or require hospitalization, may be considered a serious adverse experience when, based upon appropriate medical judgment, the event may jeopardize the participant and may require medical or surgical intervention to prevent one of the outcomes listed above. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

All SAEs will be:

- recorded on the appropriate SAE CRF
- followed through resolution by a study clinician
- reviewed and evaluated by a study clinician

*Hospitalization or prolonged hospitalization should be documented and reported as a serious adverse event, except if the hospitalization meets at least one of the following criteria:

- The hospitalization is less than 24 hours without an admission
- Hospitalization for respite care

- Hospitalization for a preexisting condition, provided that all of the following criteria are met:
    - The hospitalization was planned prior to the study or was scheduled during the study when elective surgery became necessary because of the expected normal progression of the disease.
    - The patient has not suffered an adverse event.

If the hospitalization meets any of these criteria, then it is not considered a serious adverse event.

•

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

Collection of laboratory data will be limited to those laboratory parameters that are relevant to safety, study outcome measures, and/or clinical outcome.

The toxicity tables are adapted from the Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 published by the US DHHS. A grade 3 or higher abnormality related to study agent in the table below will be defined as a SAE. For any other adverse event, we shall use the table in Section 9.2.1.

	Grade				
Adverse Event	**1**	**2**	**3**	**4**	**5**
Leukocytosis	-	-	>100,000/mm^3		
Abdominal distension	Asymptomatic; clinical or diagnostic observations only; intervention not indicated	Symptomatic; limiting instrumental ADL	Severe discomfort; limiting self care ADL	-	-
Abdominal pain	Mild	Moderate; limiting instrumental ADL	Severe pain; limiting self care ADL	-	-
Ascites	Asymptomatic; clinical or diagnostic observations only; intervention not indicated	Symptomatic; medical intervention indicated	Severe symptoms; invasive intervention indicated	Life-threatening consequences; urgent operative intervention indicated	Death
Bloating	No change in bowel function or oral intake	Symptomatic, decreased oral intake; change in bowel function	-	-	-
Colitis	Asymptomatic; clinical or diagnostic observations only; intervention not indicated	Abdominal pain; mucus or blood in stool	Severe abdominal pain; change in bowel habits; medical intervention indicated; peritoneal signs	Life-threatening consequences; urgent intervention indicated	Death
Colonic hemorrhage	Mild; intervention not indicated	Moderate symptoms; medical intervention or minor cauterization indicated	Transfusion, radiologic, endoscopic, or elective operative intervention indicated	Life-threatening consequences; urgent intervention indicated	Death
Colonic perforation	-	Symptomatic;	Severe symptoms;	Life-threatening	Death

	Grade				
Adverse Event	**1**	**2**	**3**	**4**	**5**
		medical intervention indicated	elective operative intervention indicated	consequences; urgent intervention indicated	
Colonic obstruction	Asymptomatic; clinical or diagnostic observations only; intervention not indicated	Symptomatic; altered GI function	Hospitalization indicated; elective operative intervention indicated; disabling	Life-threatening consequences; urgent operative intervention indicated	Death
Constipation	Occasional or intermittent symptoms; occasional use of stool softeners, laxatives, dietary modification, or enema	Persistent symptoms with regular use of laxatives or enemas; limiting instrumental ADL	Obstipation with manual evacuation indicated; limiting self care ADL	Life-threatening consequences; urgent intervention indicated	Death
Diarrhea	Increase of <4 stools per day over baseline; mild increase in ostomy output compared to baseline	Increase of 4 - 6 stools per day over baseline; moderate increase in ostomy output compared to baseline	Increase of >=7 stools per day over baseline; incontinence; hospitalization indicated; severe increase in ostomy output compared to baseline; limiting self care ADL	Life-threatening consequences; urgent intervention indicated	Death
Dyspepsia (uncomfortable, often painful feeling in the stomach, resulting from impaired digestion. Symptoms include burning stomach, bloating, heartburn, nausea and vomiting)	Mild symptoms; intervention not indicated	Moderate symptoms; medical intervention indicated	Severe symptoms; surgical intervention indicated	-	-
Flatulence	Mild symptoms; intervention not indicated	Moderate; persistent; psychosocial sequelae	-	-	-
Gastritis	Asymptomatic; clinical or diagnostic observations only; intervention not indicated	Symptomatic; altered GI function; medical intervention indicated	Severely altered eating or gastric function; TPN or hospitalization indicated	Life-threatening consequences; urgent operative intervention indicated	Death
Gastroparesis (A disorder characterized by an incomplete paralysis of the muscles of the stomach wall resulting in delayed emptying of the gastric contents into the small intestine.)	Mild nausea, early satiety and bloating, able to maintain caloric intake on regular diet	Moderate symptoms; able to maintain nutrition with dietary and lifestyle modifications; may need pharmacologic intervention	Weight loss; refractory to medical intervention; unable to maintain nutrition orally	-	-
Nausea	Loss of appetite without alteration in eating habits	Oral intake decreased without significant weight loss, dehydration or malnutrition	Inadequate oral caloric or fluid intake; tube feeding, TPN, or hospitalization indicated	-	-
Proctitis	Rectal discomfort, intervention not indicated	Symptoms (e.g., rectal discomfort, passing blood or mucus); medical	Severe symptoms; fecal urgency or stool incontinence; limiting self care ADL	Life-threatening consequences; urgent intervention indicated	Death

	Grade				
Adverse Event	**1**	**2**	**3**	**4**	**5**
		intervention indicated; limiting instrumental ADL			
Typhlitis (inflammation of the cecum)	-	-	Symptomatic (e.g., abdominal pain, fever, change in bowel habits with ileus); peritoneal signs	Life-threatening consequences; urgent operative intervention indicated	Death
Vomiting	1 - 2 episodes (separated by 5 minutes) in 24 hrs	3 - 5 episodes (separated by 5 minutes) in 24 hrs	>=6 episodes (separated by 5 minutes) in 24 hrs; tube feeding, TPN or hospitalization indicated	Life-threatening consequences; urgent intervention indicated	Death
Alanine aminotransferase	>ULN - 3.0 x ULN	>3.0 - 5.0 x ULN	>5.0 - 20.0 x ULN	>20.0 x ULN	-
Alkaline phosphatase	>ULN - 2.5 x ULN	>2.5 - 5.0 x ULN	>5.0 - 20.0 x ULN	>20.0 x ULN	-
Aspartate aminotransferase increased	>ULN - 3.0 x ULN	>3.0 - 5.0 x ULN	>5.0 - 20.0 x ULN	>20.0 x ULN	-
Blood bilirubin increased	>ULN - 1.5 x ULN	>1.5 - 3.0 x ULN	>3.0 - 10.0 x ULN	>10.0 x ULN	-
Creatinine increased	>1 - 1.5 x baseline; >ULN - 1.5 x ULN	>1.5 - 3.0 x baseline; >1.5 - 3.0 x ULN	>3.0 baseline; >3.0 - 6.0 x ULN	>6.0 x ULN	-
Weight loss	5 to <10% from baseline; intervention not indicated	10 - <20% from baseline; nutritional support indicated	>=20% from baseline; tube feeding or TPN indicated	-	-
Dehydration	Increased oral fluids indicated; dry mucous membranes; diminished skin turgor	IV fluids indicated <24 hrs	IV fluids or hospitalization Indicated	Life-threatening consequences; urgent intervention indicated	Death
Hyperglycemia	Fasting glucose value >ULN - 160 mg/dL; Fasting glucose value >ULN - 8.9 mmol/L	Fasting glucose value >160 - 250 mg/dL; Fasting glucose value >8.9 - 13.9 mmol/L	>250 - 500 mg/dL; >13.9 - 27.8 mmol/L; hospitalization Indicated	>500 mg/dL; >27.8 mmol/L; life-threatening consequences	Death
Hyperkalemia	>ULN - 5.5 mmol/L	>5.5 - 6.0 mmol/L	>6.0 - 7.0 mmol/L; hospitalization indicated	>7.0 mmol/L; life-threatening Consequences	Death
Hypernatremia	>ULN - 150 mmol/L	>150 - 155 mmol/L	>155 - 160 mmol/L; hospitalization indicated	>160 mmol/L; life-threatening Consequences	Death
Hypoalbuminemia	<LLN - 3 g/dL; <LLN - 30 g/L	<3 - 2 g/dL; <30 - 20 g/L	<2 g/dL; <20 g/L	Life-threatening consequences; urgent intervention indicated	Death
Hypoglycemia	<LLN - 55 mg/dL; <LLN - 3.0 mmol/L	<55 - 40 mg/dL; <3.0 - 2.2 mmol/L	<40 - 30 mg/dL; <2.2 - 1.7 mmol/L	<30 mg/dL; <1.7 mmol/L; life-threatening consequences; seizures	Death
Hypokalemia	<LLN - 3.0 mmol/L	<LLN - 3.0 mmol/L; symptomatic; intervention indicated	<3.0 - 2.5 mmol/L; hospitalization indicated	<2.5 mmol/L; life-threatening Consequences	Death
Hyponatremia	<LLN - 130 mmol/L	-	<130 - 120 mmol/L	<120 mmol/L; life-threatening consequences	Death

9.3 Reporting Procedures

Adverse events will be collected from Day 1 of study agent through a minimum of 7 days after last day of study agent. Serious Adverse Events that are related to the study agent will be reported through Day 190 follow-up.

9.3.1 Serious Adverse Events

The study clinician will complete a Serious Adverse Event Form within the following timelines:

Type of Event	To whom will it be reported:	Time Frame for Reporting	How reported?
Any internal event resulting in death that is deemed DEFINITELY related to (caused by) study participation (Note: An internal event is one that occurs in a participant enrolled in a UVa protocol.)	IRB-HSR FDA	Within 24 hours Within 7 days	IRB Online and phone call www.irb.virginia.edu/ Phone call, email and letter
Internal, Serious, Unexpected adverse event	IRB-HSR FDA	Within 7 calendar days from the time the study team received knowledge of the event. Within 15 days *Timeline includes submission of signed hardcopy of AE form.*	IRB Online www.irb.virginia.edu/ Email and letter
Unanticipated Problems that are not adverse events or protocol violations This would include a Data Breach.	IRB-HSR	Within 7 calendar days from the time the study team received knowledge of the event.	Unanticipated Problem report form. *http://www.virginia.edu/vprgs/irb/HSR_docs/Forms/Reporting_Requirements-Unanticipated_Problems.doc)*

Protocol Violations The IRB-HSR only requires that MAJOR violation be reported, unless otherwise required by the sponsor **OR** **Enrollment Exceptions**	IRB-HSR	Within 7 calendar days from the time the study team received knowledge of the event.	Protocol Violation and Enrollment Exception Reporting Form *http://www.virginia.edu/vprqs/irb/hsr_forms.html* *Go to 3rd bullet from the bottom*
Data Breach	The UVa Corporate Compliance and Privacy Office, a ITC: if breach involves electronic data- UVa Police if breach includes such things as stolen computers.	As soon as possible and no later than 24 hours from the time the incident is identified. As soon as possible and no later than 24 hours from the time the incident is identified. IMMEDIATELY.	UVa Corporate Compliance and Privacy Office- Phone 924-9741 ITC: Information Security Incident Reporting procedure, http://www.itc.virginia.edu/security/reporting.html Phone- (434) 924-7166
UVa PI HELD IND			
Life-threatening and/or fata unexpected events related or possibly related to the use of the investigational agent.	FDA	Within 7 calendar days of the study team learning of the event	Form FDA 3500A (MedWatch) or narrative
Serious, unexpected and related or possibly related adverse events	FDA	Within 15 calendar days after the study team receives knowledge of the event	Form FDA 3500A (MedWatch) or narrative
All adverse events	FDA	Annually	IND annual report

Other supporting documentation of the event may be requested by the IRB-HSR, Cancer Center DSMC and FDA and should be provided as soon as possible.

9.3.2 Regulatory Reporting for Studies Conducted Under a IND

All SAEs will be reported to the UVA IRB, UVA Cancer Center Oncore/DSMC, and FDA, as described in Section 9.3.1.

9.3.3 Reporting of Pregnancy

Pregnant patients will be excluded from the trial. Participants who become pregnant during the duration of study agent administration will be excluded but will continue to be followed daily until day 10 and at days 40, 70, and 190 by the study team.

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

AEs will be followed until resolved or considered stable. Follow-up will be performed daily for hospitalized participants and by telephone after discharge and for outpatients. For participants seen by health care providers as outpatient, the EMR will be reviewed for symptom, sign or laboratory abnormalities.

9.5 Modification of Study Agent(s)/Intervention(s) for a Participant

9.5.1 Dose / Schedule Modifications for a Participant

There will be no dose modification for a participant. Schedule of intake of study agent may be delayed if there are procedures or participant-related activities that are in conflict with the schedule of intake. In these cases, the study agent can be taken as soon as possible but should be within the same day as scheduled. There will be no make-up for missed doses.

9.6 Halting Rules

Safety findings that would temporarily suspend study agent until a safety review is convened (either routine or ad hoc) include occurrence of an unexpected adverse event or development of a SAE which cannot be attributed to other causes. The result of a safety review will guide decision as to whether the study agent (for an individual or study) should continue per protocol, proceed with caution, be further investigated, be discontinued, or be modified and then proceed. Suspension of enrollment (for a particular group or for the entire study) is another potential outcome of a safety review.

Safety review will be triggered as described in Section 11.4.

Initial safety review will be performed by the PI and the Cancer Center DSMC. All SAEs will be reported (Section 9.3.2) and reviewed in consultation with the UVA IRB.

Subsequent review of serious, unexpected and related adverse events by the PI, Cancer Center DSMC ethics review committee or IRB, the FDA, and other regulatory authorities may also result in suspension of further trial interventions/administration of study agent at a site. The FDA, other regulatory authorities, and the study sponsor(s) retain the authority to suspend additional enrollment and Study Agent(s)/Intervention(s) administration for the entire study as applicable. Theoretically, participants with liver disease may have decreased ammonia excretion and may be predisposed to the accumulation of ammonia, a breakdown product of glutamine. Similarly, patients with kidney disease may have decreased renal excretion of ammonia and glutamic acid[34]. Therefore, the following are risks that may be expected to occur in participants who have underlying liver or kidney dysfunction and taking alanyl-glutamine: elevation of liver enzymes, increased ammonia levels and increased BUN. Although we expect that these are low risk events and will have a maximum grade of 3, participants who have liver and kidney disease are excluded from the study. Enrolled subjects will be tested for ammonia levels if clinically indicated.

9.7 Stopping Rules for an Individual Participant

Criteria for Discontinuation of Study Agent(s)/Intervention(s) for Withdrawal of a Participant.

The study agent for an individual will be discontinued if there is development of laboratory toxicities, study closure due to DSMC review, or discretion of IND holder.

A study participant will be discontinued from further Study Agent if any clinical adverse event, laboratory abnormality, intercurrent illness, other medical condition or situation occurs such that continued participation in the study would not be in the best interest of the participant. Development of any exclusion criteria at the time of administration of the study agent will be cause for discontinuation.

Note that the participant will continue to be followed with participant's permission if Study Agent is discontinued. There will be no modifications to the schedule and duration of continued follow-up even if study agent is discontinued.

Discontinuation of the study will only occur if the PI, DSMC or IRB deem it necessary.

9.8 Premature Withdrawal of a Participant

Study participants may voluntarily withdraw any time after enrolment. However, with their permission, they will still be followed up at Days 40, 70 and 190 post-enrollment to gather information on recurrent diarrhea and documented CDI.

9.9 Replacement of a Participant Who Discontinues Study Treatment

Discontinued participants will not be replaced.

9.10 Safety Oversight (ISM plus SMC or DSMC)

The Cancer Center Data and Safety Monitoring Committee will be the safety oversight body for this investigator-initiated trial. Adverse events, audit results and protocol violations will be reported to the DSMC per the Cancer Center NIH approved institutional plan. The DSMC will meet every month for aggregate review of AE data. Tracking reports of the meetings are available to the PI for review. Issues of immediate concern by the DSMC are brought to the attention of the PI (and if appropriate to the PRC and IRB) and a formal response from the PI is requested.

10 CLINICAL MONITORING

10.1 Site Monitoring Plan

This clinical trial will be performed in one site only. The PI and the study coordinator shall monitor this site by reviewing 15% of all subjects randomly selected for 100% source documentation verification semiannually to determine if any breach in the protocol occurred regarding human participant protection, study procedures, laboratory, study intervention administration, and data collection processes. The principal investigator, assisted by the study coordinator, will be responsible for conducting the monitoring and will be responsible for ensuring that monitoring findings are addressed. The UVA Post Approval Monitoring (PAM) office will audit the study on a randomly selected basis.

10.2 Safety Monitoring Plan

Data and safety monitoring will be performed under the direction of the PI or her designee in this pilot study. All AEs, protocol violations and study data will be entered into Oncore/DSMC and will be reviewed by the Cancer Center Data and Safety Monitoring Committee monthly.

11 STATISTICAL CONSIDERATIONS

11.1 Study Design

This is a single arm phase II trial to determine whether the data support the underlying hypothesis that supplementation of standard CDI treatment with AG will decrease the 40-day rate of clinical failure by 50%; and to obtain preliminary estimates of secondary endpoints. Study objectives are described in section 3.2 and endpoint in section 4.1.

11.2 Sample Size Considerations

A recent clinical epidemiologic study at the UVA Health System recruited 362 participants who were admitted and diagnosed with CDI from October 2008 to August 2011. For 80% of these participants, this was their first episode of CDI. Forty-seven percent had concurrent or a history of solid organ or hematologic malignancy. Louie et al 2011 report results from a randomized trial of fidaxomicin versus vancomycin for CDI where 40-day clinical failure was observed in 36% (95% CI(31, 42%) of participants treated with vancomycin (36). For this study, target sample size is based upon detecting a 50% reduction in 40-day clinical failure (to 18%) compared to the null of 36% with a one-sided 10% significance level using an exact test. Accrual of 40 eligible participants provides approximately 90% power to test for a null 40-day clinical failure rate against the alternative of 18%. Adjusting for a 5% ineligibility rate maximum sample size is estimated at 43 participants. Accrual is estimated at 3-4 participants a month, thus accrual to the study should be completed in 4 years.

11.3 Randomization/Stratification

Randomization is not part of this single arm study.

11.4 Safety Monitoring

Safety monitoring guidelines have been generated for the study. The stopping boundary will be monitored and if a stopping boundary is crossed then accrual to the study will be suspended until the study PI, co-investigators and the DSMC can review the data, and determine if whether the study should continue, be amended or be closed to further accrual.

11.4.1 SAEs

Safety will be assessed by monitoring the number of participants who experience an SAE with AG treatment. This will be performed by the PI and Cancer Center DSMC. Results from Louie et al 2011 reported an SAE rate of approximately 24.1% (95% CI(20, 29%)) for participants treated with vancomycin. The upper boundary of a sequential

probability ratio test (SPRT) based upon a binomial test of proportions for SAE will be used for monitoring to protect against excessive SAE rates. The stopping boundary is for a SPRT contrasting an assumed SAE rate of 25% versus an unacceptable SAE rate of 35%, with nominal type I and II errors of 10% and 10%, respectively and results in a regression coefficient of 0.298. Thus, the safety boundary will be considered crossed if at any time > 28.9% of accrued participants experience a SAE

11.4.1 30 day mortality

30-day mortality (Day 40 post-enrollment) will be assessed by monitoring the number of participants who die within 40 days of starting protocol treatment. Results from Hensgens et al 2013 reported an all-cause mortality risk of 13.1% (95% CI(11, 15%)) after 30 days (37). The stopping boundary is for a SPRT contrasting an assumed 30-day mortality rate of 13% versus an unacceptable rate of 20%, with nominal type I and II errors of 10% and 10%, respectively and results in a regression coefficient of 0.163. Thus, the safety boundary will be considered crossed if at any time > 16.3% of accrued participants die within 40 days of starting protocol treatment.

11.5 Analyses

Primary analyses will be based upon intention-to-treat where all eligible participants are included in the analysis regardless of amount of treatment received or compliance. Secondary analyses will assess the impact of non-compliance to treatment if 10% or more of participants do not comply with the treatment schedule.

Adverse events and SAEs will be tabulated by frequency, severity and attribution. The one-sample exact test for proportions will be used to test the hypothesis. 90% confidence intervals for a one-sample proportion will be estimated. Point estimates and 90% confidence intervals will be calculated for clinical failure rates at 70 and 190 days post-enrollment. CDI recurrence will be estimated by a cumulative incidence curve with death as a competing risk. Duration of diarrhea will be calculated from the day the participant reports the onset of diarrhea to the day it stops. Maximum duration will be reported for multiple episodes.

The product limit method of Kaplan and Meier will be used to estimate overall survival and the Cox proportional hazards maximum likelihood estimate will be used to estimate the association of baseline factors and overall survival.

12 SOURCE DOCUMENTS AND ACCESS TO SOURCE DATA/DOCUMENTS

The site will maintain appropriate medical and research records for this trial, in compliance with ICH E6, Section 4.9 and regulatory and institutional requirements for the protection of confidentiality of participants. All participants will be coded in official record and their identities only known to the PI and selected personnel assisting the PI in enrollment, screening, and follow-up of the enrolled participants. Participants will be referred to by code only, keeping their identities confidential from outside parties. All information in which names or personal identifiers of Participants are linked to the donor code is kept on a password-protected computer device or in a locked file cabinet in the PI's office. Data arising from the project and publicly presented will be independent of any personal identifier. All clinical specimens will be coded. Personnel processing clinical specimens will not have access to the code.

This site will permit authorized representatives of 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.

Data gathered will come from the electronic medical records (EPIC) (for clinical progress), symptom diary (specific signs and symptoms), and laboratory results (clinical microbiology for screening stool tests) and research lab for sera citrulline and LPS assays, stool lactoferrin assay, clostridial PCR and toxin assay, and select microbial assay. Data will be compiled for each participant in a Case Report Form (CRF) and encoded in the database.

13 QUALITY CONTROL AND QUALITY ASSURANCE

The PI and study coordinator shall review 15% of the data semiannually and determine if any breach in the protocol occurred regarding human participant protection, study procedures, laboratory, study intervention administration, and data collection processes. During these reviews all data fields in the OnCore database will be monitored. The principal investigator, assisted by the study coordinator, will be responsible for conducting the review of the data source and database. All research personnel will be required to complete CITI training.

See Section 10.1 for detail on Site Monitoring.

14 ETHICS/PROTECTION OF HUMAN PARTICIPANT S

14.1 Ethical Standard

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

14.2 Institutional Review Board

The study protocol will be reviewed and approved by the Cancer Center PRC and the UVA IRB. Any amendments to the protocol consent materials must also be approved before they are placed into use.

14.3 Informed Consent Process

Informed consent is a process that is initiated prior to the individual's agreeing to participate in the study and continuing throughout the individual's study participation. Extensive discussion of risks and possible benefits of this therapy will be provided to the participants and their families. Consent forms describing in detail the study interventions/products, study procedures, and risks are given to the participant and written documentation of informed consent is required prior to starting intervention/administering study product. Consent forms will be IRB-approved and the participant will be asked to read and review the document. Upon reviewing the document, the investigator will explain the research study to the participant and answer any questions that may arise. The participants will sign the informed consent document prior to any procedures being done specifically for the study. The participants should have the opportunity to discuss the study with their surrogates or think about it prior to agreeing to participate. The participants may withdraw consent at any time throughout the course of the trial. A copy of the signed informed consent document will be given to the participants for their records. The rights and welfare of the participants 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. If the participant has cognitive impairment, consent will be deferred to the patient's surrogate.

14.4 Subject Confidentiality

Participant confidentiality is strictly held in trust by the participating investigators and staff. This confidentiality is extended to cover testing of biological samples and genetic tests in addition to the clinical information relating to participating participants.

The study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorized third party.

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

14.5 Study Discontinuation

In the event that the study is discontinued, all participants will continue their standard therapy as prescribed by their health care provider or team. There will be no crossover to study drug for placebo recipients at the completion of the study.

14.6 Future Use of Stored Specimens

Residual specimens will be maintained after the study is complete. The consent form will include storage of specimens for future studies, for which the participant may opt out from. The specimens will not contain any participant identifiers and will be maintained at UVA only. If shipped for further testing elsewhere, the receiving institution will ship back or dispose leftover specimens after appropriate processing. These specimens will be coded and delinked from the medical records at the completion of the study, data analyses and publication of results. The IRB will review future studies, and protections of confidentiality for any future studies with the stored specimens, eg, specimens will be coded, bar-coded, delinked. Unless approved by the IRB, genetic testing will not be performed.

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 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 documents and maintained for recording data for each participant enrolled in the study. Data reported in the eCRF derived from source documents should be consistent with the source documents or the discrepancies should be explained

15.1 Data Management Responsibilities

All source documents and laboratory reports must be reviewed by the clinical team and data entry staff, who will ensure that they are accurate and complete. Adverse events must be graded, assessed for severity and causality, and reviewed by the PI or designee.

Data collection is the responsibility of the clinical trial staff under the supervision of the PI. During the study, the investigator must maintain complete and accurate documentation for the study.

The PI, study coordinator, and the biostatistician 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, interventions, and other clinical data) and clinical laboratory data coming from EPIC, participant symptoms and follow-up information from the symptom diary and the results of specimen testing from the research laboratory will all be entered into Oncore as required by the Cancer Center. The data system includes password protection and internal quality 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 (hematologic, chemistry and microbiologic), and outcome measures (eg, duration of diarrhea, adverse events, recurrence, and death).

15.4 Timing/Reports

Daily clinical data will be reviewed while inpatient participants are in the hospital and receiving study agent through Day 10. A semi-annual review of CRFs and source documents will be performed as described in Sections 10, 11 and 13. Stopping rules and reports are described in Section 9. The PI will be reviewing the study for data and safety semi-annually. The Cancer Center DSMC reviews data and safety monthly and reports annually. An interim analysis for adverse events will be performed half-way through enrollment.

15.5 Study Records Retention

Study documents should be retained for a minimum of 6 years after the completion of the study. These documents should be retained for a longer period, however, if required by local regulations.

15.6 Protocol Deviations

A protocol deviation is any noncompliance with the clinical trial protocol, Good Clinical Practice (GCP), or Manual of Procedures requirements. The noncompliance may be either on the part of the participant, the investigator, or the study site staff. As a result of deviations, corrective actions are to be developed 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 from the protocol must be addressed in study participant source documents. A completed copy of the Protocol Deviation Form must be maintained in the regulatory file, as well as in the participant's source document. Protocol deviations must be sent to the IRB per their guidelines. The site PI/study staff is responsible for knowing and adhering to the IRB requirements.

16 PUBLICATION POLICY

Following completion of the study, the investigator is expected to publish the results of this research in a scientific journal. The International Committee of Medical Journal Editors (ICMJE) member journals have adopted a trials-registration policy as a condition for publication. This policy requires that all clinical trials be registered in a public trials registry such as ClinicalTrials.gov*, which is sponsored by the National Library of Medicine. Other biomedical journals are considering adopting similar policies. It is the responsibility of the overall PI to register this trial in an acceptable registry. Any clinical trial starting enrollment after 01 July 2005 must be registered on or before participant enrollment

The ICMJE defines a clinical trial as any research project that prospectively assigns human participants to intervention or comparison groups to study the cause-and-effect relationship between a medical intervention and a health outcome. Studies designed for other purposes, such as to study pharmacokinetics or major toxicity (eg, Phase I trials), would be exempt from this policy.

*Journal Citation:

De Angelis C, Drazen JM, Frizelle FA, Haug C, Hoey J, Horton R, et al. Clinical trial registration: a statement from the International Committee of Medical Journal Editors. N Engl J Med. 2004;351:1250-1.

17 LITERATURE REFERENCES

1. Ziegler TR, Benfell K, Smith RJ, et al. Safety and metabolic effects of L-glutamine administration in humans. *JPEN J Parenter Enteral Nutr*. 1990;14(4 Suppl):137S-146S.

2. Jian ZM, Cao JD, Zhu XG, et al. The impact of alanyl-glutamine on clinical safety, nitrogen balance, intestinal permeability, and clinical outcome in postoperative patients: A randomized, double-blind, controlled study of 120 patients. *JPEN J Parenter Enteral Nutr*. 1999;23(5 Suppl):S62-6.

3. Garlick PJ. Assessment of the safety of glutamine and other amino acids. *J Nutr*. 2001;131(9 Suppl):2556S-61S.

4. Fürst P, Alteheld B, Stehle P. Why should a single nutrient-glutamine-improve outcome?: The remarkable story of glutamine dipeptides. *Clinical Nutrition Supplements*. 2004;1(1):3-15.

5. Harris RC, Hoffman JR, Allsopp A, Routledge NB. L-glutamine absorption is enhanced after ingestion of L-alanylglutamine compared with the free amino acid or wheat protein. *Nutr Res*. 2012;32(4):272-277. doi: 10.1016/j.nutres.2012.02.003; 10.1016/j.nutres.2012.02.003.

6. Lacey JM, Wilmore DW. Is glutamine a conditionally essential amino acid? *Nutr Rev*. 1990;48(8):297-309.

7. Rhoads JM, Argenzio RA, Chen W, et al. L-glutamine stimulates intestinal cell proliferation and activates mitogen-activated protein kinases. *Am J Physiol*. 1997;272(5 Pt 1):G943-53.

8. Wischmeyer PE, Musch MW, Madonna MB, Thisted R, Chang EB. Glutamine protects intestinal epithelial cells: Role of inducible HSP70. *Am J Physiol*. 1997;272(4 Pt 1):G879-84.

9. Papaconstantinou HT, Hwang KO, Rajaraman S, Hellmich MR, Townsend CM,Jr, Ko TC. Glutamine deprivation induces apoptosis in intestinal epithelial cells. *Surgery*. 1998;124(2):152-9; discussion 159-60.

10. Klimberg VS, Salloum RM, Kasper M, et al. Oral glutamine accelerates healing of the small intestine and improves outcome after whole abdominal radiation. *Arch Surg*. 1990;125(8):1040-1045.

11. Scheppach W, Dusel G, Kuhn T, et al. Effect of L-glutamine and n-butyrate on the restitution of rat colonic mucosa after acid induced injury. *Gut*. 1996;38(6):878-885.

12. Fujita T, Sakurai K. Efficacy of glutamine-enriched enteral nutrition in an experimental model of mucosal ulcerative colitis. *Br J Surg*. 1995;82(6):749-751.

13. Ersin S, Tuncyurek P, Esassolak M, et al. The prophylactic and therapeutic effects of glutamine- and arginine-enriched diets on radiation-induced enteritis in rats. *J Surg Res*. 2000;89(2):121-125. doi: 10.1006/jsre.1999.5808.

14. Chun H, Sasaki M, Fujiyama Y, Bamba T. Effect of enteral glutamine on intestinal permeability and bacterial translocation after abdominal radiation injury in rats. *J Gastroenterol*. 1997;32(2):189-195.

15. Erbil Y, Berber E, Ozarmagan S, et al. The effects of sodium deoxycholate, lactulose and glutamine on bacterial translocation in common bile duct ligated rats. *Hepatogastroenterology*. 1999;46(29):2791-2795.

16. Ziegler TR, Mantell MP, Chow JC, Rombeau JL, Smith RJ. Gut adaptation and the insulin-like growth factor system: Regulation by glutamine and IGF-I administration. *Am J Physiol*. 1996;271(5 Pt 1):G866-75.

17. Brito GA, Carneiro-Filho B, Oria RB, Destura RV, Lima AA, Guerrant RL. Clostridium difficile toxin A induces intestinal epithelial cell apoptosis and damage: Role of gln and ala-gln in toxin A effects. *Dig Dis Sci*. 2005;50(7):1271-1278.

18. Santos AA, Braga-Neto MB, Oliveira MR, et al. Glutamine and alanyl-glutamine increase RhoA expression and reduce clostridium difficile toxin-a-induced intestinal epithelial cell damage. *Biomed Res Int*. 2013;2013:152052. doi: 10.1155/2013/152052; 10.1155/2013/152052.

19. Warren CA, Calabrese GM, Li Y, et al. Effects of adenosine A(2)A receptor activation and alanyl-glutamine in clostridium difficile toxin-induced ileitis in rabbits and cecitis in mice. *BMC Infect Dis*. 2012;12:13-2334-12-13. doi: 10.1186/1471-2334-12-13; 10.1186/1471-2334-12-13.

20. Carneiro BA, Fujii J, Brito GA, et al. Caspase and bid involvement in clostridium difficile toxin A-induced apoptosis and modulation of toxin A effects by glutamine and alanyl-glutamine in vivo and in vitro. *Infect Immun*. 2006;74(1):81-87. doi: 10.1128/IAI.74.1.81-87.2006.

21. Warren CA, van Opstal EJ, Riggins MS, et al. Vancomycin treatment's association with delayed intestinal tissue injury, clostridial overgrowth, and recurrence of clostridium difficile infection in mice. *Antimicrob Agents Chemother*. 2013;57(2):689-696. doi: 10.1128/AAC.00877-12; 10.1128/AAC.00877-12.

22. Rodrigues RS, Oliveira RA, Li Y, et al. Intestinal epithelial restitution after TcdB challenge and recovery from clostridium difficile infection in mice with alanyl-glutamine treatment. *J Infect Dis*. 2013;207(10):1505-1515. doi: 10.1093/infdis/jit041; 10.1093/infdis/jit041.

23. Sufit A, Weitzel LB, Hamiel C, et al. Pharmacologically dosed oral glutamine reduces myocardial injury in patients undergoing cardiac surgery: A randomized pilot feasibility trial.

JPEN J Parenter Enteral Nutr. 2012;36(5):556-561. doi: 10.1177/0148607112448823; 10.1177/0148607112448823.

24. Leite RD, Lima NL, Leite CA, Farhat CK, Guerrant RL, Lima AA. Improvement of intestinal permeability with alanyl-glutamine in HIV patients: A randomized, double blinded, placebo-controlled clinical trial. *Arq Gastroenterol*. 2013;50(1):56-63.

25. Bushen OY, Davenport JA, Lima AB, et al. Diarrhea and reduced levels of antiretroviral drugs: Improvement with glutamine or alanyl-glutamine in a randomized controlled trial in northeast brazil. *Clin Infect Dis*. 2004;38(12):1764-1770. doi: 10.1086/421394.

26. Heiser CR, Ernst JA, Barrett JT, French N, Schutz M, Dube MP. Probiotics, soluble fiber, and L-glutamine (GLN) reduce nelfinavir (NFV)- or lopinavir/ritonavir (LPV/r)-related diarrhea. *J Int Assoc Physicians AIDS Care (Chic)*. 2004;3(4):121-129.

27. Savarese D, Al-Zoubi A, Boucher J. Glutamine for irinotecan diarrhea. *J Clin Oncol*. 2000;18(2):450-451.

28. Stubblefield MD, Vahdat LT, Balmaceda CM, Troxel AB, Hesdorffer CS, Gooch CL. Glutamine as a neuroprotective agent in high-dose paclitaxel-induced peripheral neuropathy: A clinical and electrophysiologic study. *Clin Oncol (R Coll Radiol)*. 2005;17(4):271-276.

29. Wang WS, Lin JK, Lin TC, et al. Oral glutamine is effective for preventing oxaliplatin-induced neuropathy in colorectal cancer patients. *Oncologist*. 2007;12(3):312-319. doi: 10.1634/theoncologist.12-3-312.

30. Lucado J, Gould C, Elixhauser A. Clostridium difficile infections (CDI) in hospital stays, 2009: Statistical brief #124. In: *Healthcare cost and utilization project (HCUP) statistical briefs.* Rockville (MD): ; 2006.

31. Centers for Disease Control and Prevention (CDC). Vital signs: Preventing clostridium difficile infections. *MMWR Morb Mortal Wkly Rep*. 2012;61(9):157-162.

32. Heyland D, Muscedere J, Wischmeyer PE, et al. A randomized trial of glutamine and antioxidants in critically ill patients. *N Engl J Med*. 2013;368(16):1489-1497. doi: 10.1056/NEJMoa1212722; 10.1056/NEJMoa1212722.

33. Hornsby-Lewis L, Shike M, Brown P, Klang M, Pearlstone D, Brennan MF. L-glutamine supplementation in home total parenteral nutrition patients: Stability, safety, and effects on intestinal absorption. *JPEN J Parenter Enteral Nutr*. 1994;18(3):268-273.

34. Sacks GS. Glutamine supplementation in catabolic patients. *Ann Pharmacother*. 1999;33(3):348-354.

35. Cohen SH, Gerding DN, Johnson S, et al. Clinical practice guidelines for clostridium difficile infection in adults: 2010 update by the society for healthcare epidemiology of america (SHEA) and the infectious diseases society of america (IDSA). *Infect Control Hosp Epidemiol*. 2010;31(5):431-455. doi: 10.1086/651706; 10.1086/651706.

36. Louie TJ, Miller MA, Mullane KM, et al. Fidaxomicin versus vancomycin for *Clostridium difficile* infection. *New Engl J Med* 2011:364:422-31.

37. Hensgens MPM, Goorhius A, Dekkers OM, et al. All-cause and disease-specific mortality in hospitalized patients with *Clostridium difficile* infection: a multicenter cohort study. *Clin Infect Dis* 2013;56(8):1108-16.

SUPPLEMENTS/APPENDICES

APPENDIX A: SCHEDULE OF EVENTS

				Follow-Up Schedule								
Procedures		Screening	Baseline/Day 0	Days 1-4	Day 5	Days 6-9	Day 10+ 2	Day 40 +/- 7	Day 70 +/- 7	Day 190 +/- 7	Study Completion	Premature Discontinuation
Signed Consent Form		X	X									
Assessment of Eligibility Criteria		X	X									
Review of Medical History			X									
Review of Concomitant Medications			X	X	X	X	X	X	X	X	X	X
Study Intervention			X	X	X	X	X					
Clinical Monitoring and Follow-Up			X	X	X	X	X	X	X	X	X	X
Assessment of Adverse Events				X	X	X	X	X	X	X	X	X
Clinical Laboratory	Chemistry		X									
	Hematology		X									
	Stool	X										
Research Laboratory												
	Stool[a]	X										

[a]This specimen will come from the clinical laboratory and no additional collection will be performed if not available.

UVA Clinical Laboratory

Hematology –White blood cell, Red blood cell, Hemoglobin, Hematocrit, platelet count

Biochemistry – Sodium, potassium, chloride, bicarbonate, BUN, creatinine, glucose, albumin, total bilirubin, alkaline phosphate, AST, ALT, erythrocyte sedimentation rate, C-reactive protein, serum pregnancy test (HCG qualitative)

UVA Clinical Microbiology Laboratory

Stool: *C. difficile* toxin B PCR

Warren Research Laboratory at UVA

Stool: Lactoferrin assay, *C. difficile* toxin B PCR, *C. difficile* toxin A and B ELISA, anaerobic culture and ribotyping of isolated C*. difficile*