A clinical trial for the off-label use of ursodiol for the prevention of recurrent C. difficile colitis and diarrhea (2).

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List of Abbreviations

Ursodeoxycholic acid urso, ursodiol

C. difficile *clostridium difficile*

Study Summary

Title	*A clinical trial for the off-label use of ursodiol for the prevention of recurrent C. difficile colitis and diarrhea.*
Short Title	*C. difficile and Urso.*
Protocol Number	*S15-01348*
Phase	*Clinical trial of an off-label use of ursodeoxycholic acid*
Methodology	*Open label study with fecal bile acid monitoring*
Study Duration	*10 months*
Study Center(s)	*Multi-center Tisch (including NYU Lutheran sites),Bellevue Hospital*
Objectives	*Prevention of recurrent C. difficile colitis and diarrhea*
Number of Subjects	*32 enrolled to reach goal of 30 completed subjects*
Diagnosis and Main Inclusion Criteria	*Suspected C. difficile colitis and diarrhea*
Study Product, Dose, Route, Regimen	*Ursodiol 300 mg by mouth twice a day; added to Metronidazole and/or vancomycin therapy*
Duration of administration	*Two months*
Reference therapy	*Metronidazole and/or Vancomycin*
Statistical Methodology	*Percent of patients with restoration of deoxycholic acid pool size*

1 Introduction

This document is a clinical research protocol with each patient serving as his/her own control. Good Clinical Practice Standards will be followed in accordance with US government research regulations, and applicable international standards of Good Clinical Practice, and institutional research policies and procedures.

1.1 Background

The occurrence of inflammation of the colon and diarrhea after a patient has received a therapeutic course of antibiotics for an unrelated problem (i.e. pneumonia, urinary tract infection) is a well-recognized clinical problem. In many instances, it has been found that when the feces of the patient undergo bacterial culture, they contain the bacterium clostridium difficile, which is known to produce a toxin that causes colonic inflammation and diarrhea. Hence the name *C. difficile* colitis and/or diarrhea. Data collected in 2011 indicates that the burden of *C. difficile* infection in the United States is estimated as 15,463 cases per year with first recurrences of 83,000 cases per year and estimated number of deaths as 29,300 per year.(3)

The pathogenesis of this post-antibiotic *C. difficile* colitis has now been identified (See Figures 1a and 1b) and is outlined as follows:

a. Antibiotics suppress the growth of the normal intestinal bacterial flora
    1. These bacteria have enzymes that normally metabolize the conjugated bile acids made by the liver, secreted into bile and emptied into the intestines, (particularly the glycine and taurine conjugates of cholic acid (80%)) to unconjugated cholic acid.
    2. Other normal bacterial flora have enzymes that metabolize only the unconjugated cholic acid to deoxycholic acid, which normally account for 80% of total fecal bile acids.
b. Loss of these normal bacterial flora results in an increase in conjugated bile acids and absence of deoxycholic acid in the colon.
c. Loss of deoxycholic acid permits the growth of *C. difficile* and toxin production with initiation of colitis and diarrhea.

Figure 1A.

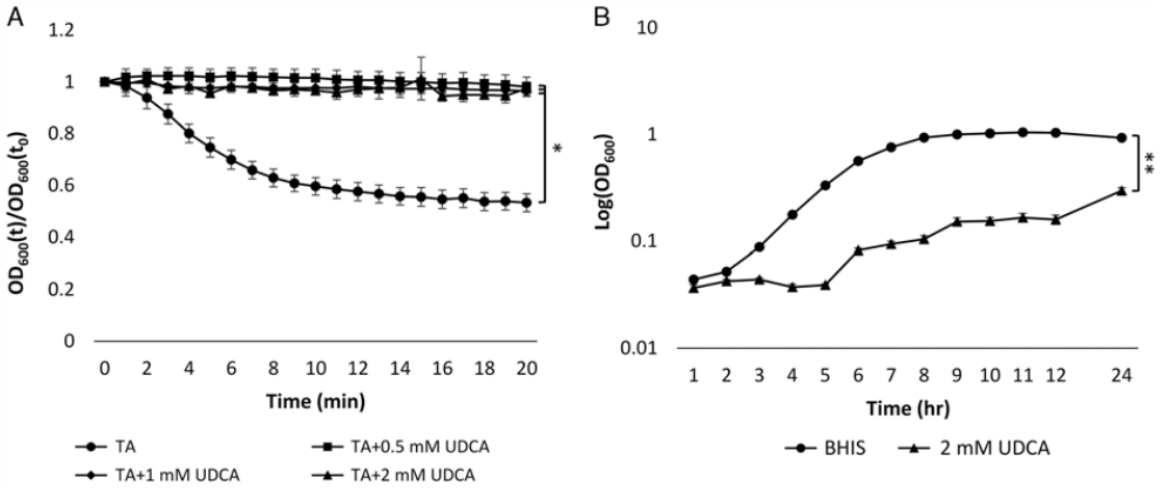

FIGURE 1. UDCA inhibits the germination and the growth of *Clostridium difficile* isolated from patients' stool. A, The relative OD_{600} of spores isolated from the patient exposed to 0.5 mM (■), 1 mM (♦), or 2 mM (▲) UDCA in the presence of 2 mM TA versus 2 mM TA alone (●). $OD_{600}(t)/OD_{600}(t_0) = OD_{600}$ normalized to the initial OD_{600} (relative OD_{600}). B, Growth of vegetative cells from a *C. difficile* isolate in BHIS alone (●) or BHIS with 2 mM UDCA (▲). $^*P < 0.01$, $^{**}P < 0.0001$. Data represent mean ± SEM. TA indicates taurocholate; UDCA, ursodeoxycholic acid.

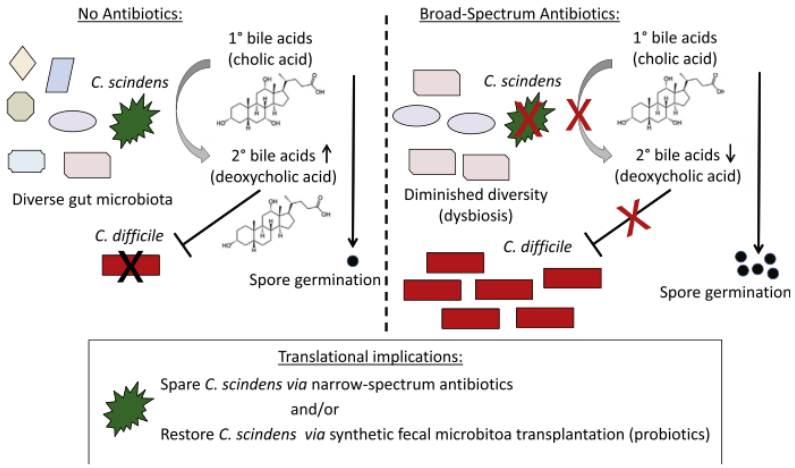

Figure 1. *S. scindens* and Secondary Bile Acids Inhibit *C. difficile* Growth
(Left) In the absence of antibiotics, the gut microbial community is diverse and includes bacteria such as *S. scindens* that convert primary bile acids into secondary bile acids, which, in turn, inhibits the growth of *C. difficile*. (Right) Broad-spectrum antibiotics diminish the diversity of the gut microbial community. Loss of certain bacteria such as *C. scindens* results in reduced production of secondary bile acids and increased growth of *C. difficile*. Additionally, accumulation of primary bile acids leads to increased spore germination of *C. difficile*.

Figure 1B

1.2 Non-Investigational and Investigational Agent

The non-investigational reference therapies, metronidazole, vancomycin and fidaxomicin , are FDA-approved antibiotics currently in use today because of their effectiveness in suppressing the growth of *C. difficile* and therefore quickly eliminating the colitis and diarrhea. HOWEVER, they do not promote the restoration of the normal bacterial flora. THEREFORE, when medication is stopped, the *C. difficile* colitis returns. Hence the name recurrent *C. difficile* colitis and diarrhea. A more recent FDA approved approach is fecal microbiota transplant (FMT) where the patient is given prepared fecal material from a normal donor. This successful approach is based on the concept that it provides normal enteric flora which presumably restore normal bile acid metabolism. Refractoriness to these approaches occasionally results in removal of the diseased colon (4).

Based on the success of FMT together with the knowledge that deoxycholic acid (1) and more recently ursodeoxycholic acid (2) suppress the growth of C. difficile, it is reasonable to test the hypothesis that augmenting the FDA approved antibiotic therapy with co-administration of ursodeoxycholic acid may be an alternate approach for the restoration of normal enteric flora.

Success of FMT therapy was based on the absence of recurrence of symptoms of CDI within 8 weeks (5). The highest success rate was achieved by direct transplantation into the colon (5). Administration of ursodeoxycholic acid for 2 months will test whether it is a surrogate for FMT with respect to recurrence of symptoms. In addition, because we are analyzing fecal bile acids at 2 week intervals we will know in what percentage of patients there has been restoration of the endogenous deoxycholic acid pool and approximate rates at which restoration occurs. This information will provide guidance for future studies.

Considering the complexity of the problems that have arisen with FMT therapy (5), it is reasonable to propose that the proposed study can identify individuals that restore their endogenous pool and do not require FMT therapy.

Ursodeoxycholic acid (250mg and 500mg tablets) was approved by the FDA more than 15 years ago for the treatment of primary biliary cirrhosis. The approved dose is 13 to 15 mg/kg/day in 2 to 4 divided doses. Additionally, it is indicated for prevention of gallstones in patients undergoing rapid weight loss at a dose of 600mg/day (300mg capsules twice a day) and for treatment of radiolucent gallbladder stones (8-10mg/kg/day in 2 or 3 divided doses). Except for occasional diarrhea, there is no acute toxicity.

Ursodiol is a naturally occurring bile acid found in small quantities in normal human bile. It is a bitter-tasting white powder insoluble in water but soluble in ethanol and other organic solvents. The chemical name is 3α,7β–dihydroxy-5β–cholan-24-oic acid with a molecular weight of 392.58. It is stable at room temperature for at least a year.

About 90% of a therapeutic dose of ursodiol is absorbed from the small intestines after one administration. It undergoes efficient extraction from portal vein blood by the liver (large "first-pass" effect) and is conjugated with glycine or taurine before undergoing excretion into bile. Only small amounts of Ursodiol enter the systemic circulation and very little is excreted in urine. The taurine and glycine conjugates of ursodiol undergo intestinal reabsorption by transporters in the ileum together with other conjugated bile acids. Administered ursodiol that is not absorbed enters the colon together with small amounts of the conjugates which are hydrolyzed and are metabolized to lithocholic acid.

Lithocholic acid can cause cholestatic liver disease and cirrhosis in non-human species that cannot sulfate lithocholic acid. Humans have the capacity to sulfate lithocholic acid and liver injury has not been associated with ursodiol therapy. Although diminished capacity to sulfate because of underlying liver disease may exist such a deficiency in humans has not been clearly demonstrated.

We expect that the off-label use that we are proposing (investigational agent) will not be longer than 2 months. The FDA has granted IND Exemption for this clinical investigation.

1.3 Preclinical Data

We are only aware of the one study in one patient published in the Journal of Clinical Gastroenterology this year identifying clinical efficacy. However the basic studies, done in vitro, that support its clinical use are so compelling we believe a clinical trial is fully merited. Also a study in mice and humans (6) established that antibiotics suppress the growth of *Clostridium scindans*, a bile acid 7-dehydroxylating intestinal bacterium essential for generating deoxycholic acid, thus permitting the growth of C. difficile. Restoration of intestinal *C. scindans* restored the resistance to *C. difficile* infection.

1.4 Clinical Data to Date:

A recent clinical study (reference 2) was published concerning the off-label use of ursodeoxycholic acid (ursodiol) in a single patient with recurrent *C. difficile* "pouchitis" who had been receiving repeated courses of antibiotics. On the basis of encouraging data from in-vitro studies, oral ursodiol (300mg twice daily) was added to the patient's regimen for two weeks and well-tolerated. After 2 weeks, the urosodiol dose was increased to 300mg 4 times daily (20 mg/kg/d) and oral vancomycin (125mg/d) was discontinued.

The frequency of diarrhea decreased and firm bowel movements were reported. Stool cultures were negative for *C. difficile* by PCR tests for toxin B for over 12 months following the discontinuation of vancomycin. Follow-up endoscopic examination of the pouch at 6 months and 1 year showed no signs of inflammation. The ursodiol dose was decreased to 300mg twice daily at 7 months without relapse of *C. difficile* infection. The patient refused to discontinue ol altogether in fear of relapse of *C. difficile* infection and no adverse effects have occurred, consistent with the knowledge that has accumulated with the use of ursodiol for FDA approved indications.

In addition, in vitro findings in this study (see figure 2) establish that ursodeoxycholic acid is a surrogate for deoxycholic acid in preventing the growth of *C. difficile*, thus explaining why the recurrence was interrupted and the patient cured. On the basis of these encouraging in vitro results, the patient was prescribed oral UCDA (300 mg twice daily), which was well tolerated. After 2 weeks, the UDCA dose was increased to 300 mg 4 times daily (20 mg/kg/d) and oral vancomycin (125 mg/d) was discontinued. The frequency of diarrhea decreased and firm bowel movements were reported. Furthermore, the stool remained negative for *C. difficile* by PCR tests for toxin B over 12 months after the termination of vancomycin. Endoscopic examination of the pouch at 6 months and 1 year showed no signs of

inflammation. The UDCA dose was decreased to 300mg twice daily at 7 months without relapse of CDI. The patient refused to discontinue UDCA altogether in fear that symptoms would come back.

1.5 Dose Rationale

The major hypothesis that we are testing is that after stopping antibiotics, urso will continue to suppress the growth of *C. difficile* until the return of the normal intestinal pool of deoxycholic acid.

Implicit in this hypothesis is the knowledge that ursodeoxycholic acid, in contrast to cholic or chenodeoxycholic acid does NOT downregulate primary bile acid synthesis in the liver (7) so that as normal enteric bacteria begin to flourish, the primary bile acids will be metabolized to intestinal bile acids and the deoxycholic acid pool will return to normal.

There are large variations in the data concerning fecal bile acid excretion in humans, but a value of 500-600 mg per day for deoxycholic acid fits well into the range. Therefore, we will use 300 mg of urso twice a day (8.57 mg/kg/day for 2 months to mimic the 8 week follow-up period in previous FMT studies (5)) during which the absence of recurrent symptoms was considered an indication of success. Also, considering the quick therapeutic response to FMT, it is reasonable to think evidence for endogenous production of deoxycholic acid should be obtained within this time frame. This dose does not exceed current recommended dosages for approved indications.

1.6 Research Risks & Benefits

1.6.1 Risk of Study Drug: For the length of time being administered and the dose prescribed, we are not aware of a toxilogic risk. However, loose stool may occur and there is the risk of an allergic reaction, although not common. Accidental aspiration of the pill while taking it, cannot be eliminated.

1.6.2 Other Risks of Study Participation: During the course of the study the patient will be required to collect stool specimens at two week intervals beginning at the time of initiating antibiotic therapy. As a secondary aim we will be testing stool for other factors that may influence *C. difficile* infection, including microbial composition via 16S rRNA sequencing and stool pH. These analyses will not reveal genetic data representative of the stool donor. Therefore there are no risks associated with genetic testing to the study subjects. No endoscopic procedures are required for participation in the study. Loss of confidentiality is a small, but potential risk; however, data is coded without identifying information and stored on a network drive protected by the NYU Langone Medical Center .

1.6.3 Potential benefits

The hypothesis we are testing is that continued administration of urso will maintain suppression of *C. difficile* while allowing for return of the normal deoxy pool and thus prevent recurrence of colitis and diarrhea. Benefit cannot be guaranteed as it may work in some patients but not others. The knowledge gained from this study may ultimately benefit future patients with recurrent *C. difficile* colitis.

Because we will be analyzing fecal bile acids every two weeks, the data can be correlated with the clinical response.

2 Study Objective

Primary Objective

To eliminate recurrent *C. difficile* colitis and diarrhea

Secondary Objectives
To learn more about the restoration of normal fecal bile acid patterns after exposure to antibiotics
To learn more about the relationship between fecal bile acids and gut environmental factors that may also predispose to *C. difficile* infection, including microbiome composition and stool pH.

3 Study Design

3.1 General Design

This is a clinical trial where fecal bile acid and microbiome composition is monitored at two week intervals for a maximum of two months in each patient beginning on entry into the study. Patients will be identified through referral from their clinical provider(s). Eligible patients will be those with a suspected diagnosis of recurrent *C. difficile* colitis (more than one episode). When possible, patients will be referred and enrolled prior to collecting a fecal specimen for *C. difficile* testing. The referring physician or a study team member will offer the patient the opportunity of participating in the clinical trial. A study team member will administer the consent, and if the patient approves and signs the consent, the clinically indicated fecal specimen will be tested for *C. difficile* as well as kept for study purposes. If the laboratory test comes back positive for *C. difficile*, the ursodiol, in addition to the standard care antibiotic, is started. If the laboratory test comes back negative, the patient's study participation will be stopped after the single stool collection. This stool specimen will serve as a negative control. For patients, who are not referred in time to collect a pre-testing fecal sample, the patient will only be enrolled if *C. difficile* infection is confirmed on clinical testing.

For patients testing positive for CDI, decisions on the antibiotic treatment will be managed by the patient's clinician, but the ursodiol will be continued for a total of 2 months. Serial collection of feces at 2 week intervals are expected to indicate an increase in ursodeoxycholic acid and the appearance of deoxycholic acid. Further increase in the deoxy pool size and obtaining a normal unconjugated/conjugated bile acid ratio (corrected for the administered urso) are anticipated to ensue within two months. Although not a mandatory part of the study, it will be of additional value to establish that the return of deoxycholic acid extends beyond the time that the patient was taking ursodiol. Therefore, in those patients that agree to be contacted and are available after completion of the study, we will ask for two additional stool samples.
At each study fecal collection, the study subjects will also be asked to complete a general healthy and dietary survey.

For each follow up visit, a -4 day window will be used to account for potential scheduling difficulties and limit the chances of running out of medication. The patient will have at most 8 pills when returning for their follow-up visit. During this follow-up visit, the patient will take back their previously dispensed bottle, as well as a newly dispensed bottle, to take home. The patient will be instructed to continue their previously dispensed bottle of ursodiol until they are finished with the original bottle. Afterwards, patients can then start their newer bottle. The patients must bring a stool sample when obtaining the next bottle of medicine.

3.2 Primary Study and Safety Endpoints

The primary study endpoint is the restoration of the deoxycholic acid pool and conjugated/unconjugated fecal bile acid pattern.

The primary clinical endpoint is no further occurrence of diarrhea or other symptoms of *C. difficile* colitis during the two month period. The subject will be evaluated by a study team member at 2 week intervals at which time a brief interim history related to the possible recurrence of symptoms of CDI is obtained. The subject will be instructed to report immediately to the referring provider, as well as the study team should symptoms possibly related to CDI occur at any time, and a further clinical evaluation will be required to

ascertain the cause of these symptoms. Patients with known lactose intolerance will be reminded of the importance of maintaining a lactose-free diet.

3.3 Secondary Study Endpoints

In addition, we will be testing stool for other gut environmental characteristics that may affect one's predisposition for *C. difficile* infection. This include testing samples for stool pH , as well as for microbiome composition. It has been proposed that CDI are associated with alkaline stool pH and that FMT resolves recurrent CDI by restoration of the normal gut flora. Therefore evaluating stool pH and microbial composition from stool samples obtained during the study period will allow the study team to determine whether or not co-administration of Ursodial with antibiotics affects these secondary endpoints as well.For all stool testing, aliquots of feces will be analyzed and the bulk sample remain frozen at -80C for possible further study related to our further understanding of *C. difficile* infection. This is optional at enrollment and the subject will always have the opportunity of specifying that stool specimens not used during the study be discarded at the end of the study rather than preserved. This decision will not affect participation. Samples will be banked in the study team's laboratory (Bellevue Hospital C & D 600/611 or the Veterans Affairs Medical Center, Manhattan Campus, 6th floor). Stored samples will be coded with the specimen ID so that subjects cannot be identified. The possibility that confidentiality could inadvertently be breached when specimens are in storage will be indicated but the design is that only the study team personnel will have the information linking the specimen to a specific subject. Samples will be stored in containers that are marked with a de-identified alphanumeric study ID, but will not contain any PHI.

We will also perform microbiome analyses by processing extracted DNA samples from collected stool for 16S rRNA. This will allow us to confirm the presence of, and evaluate the relative abundance of, *C. difficile* from study patients.

3.4 Inclusion Criteria

- Male and female patients with presumed recurrent *C. difficile* colitis
- 18 years of age and older
- Capable of giving informed consent.

There are no exclusions by race, ethnicity or sexual preference.

3.5 Exclusion Criteria

- Patients with other gastrointestinal problems prone to cause diarrhea if they cannot be controlled for the period of the study. Lactose intolerance or gluten enteropathy are not an exclusion provide that the potential subject is asymptomatic and can be expected to adhere to the appropriate dietary regimen.

- Patients with contraindications to metronidazole, vancomycin, fidaxomicin, and/or ursodiol tablets or components of the formulations.

- Patients testing positive for *C. difficile* infection who are not available for long-term follow-up (2 months) by their physician will be excluded from the study.

- Patients who will not be treated with antibiotics for the *C. difficile* infection, but will be receiving fecal transplantation instead.

Although ursodeoxycholic acid is used for the treatment of cholestasis of pregnancy (last trimester) its effects on the fetus at other times have not been studied, and the FDA does not encourage its use in

pregnancy. Therefore, pregnant women confirmed by review of clinical records will also be excluded from this study.

3.6 Subject Recruitment and Screening

Providers will identify potential subjects from patients under their care for acute illness during routine clinical visits.

If the patient has had a history of *C. difficile* infection, and is presumed to have the infection again, the provider will refer the subject to a study team member who will discuss the details of the study, risks, and benefits with potential subjects, answer any questions, and obtain written informed consent from subjects. If the subject consents, a portion of their stool sample that is clinically indicated for *C. difficile* infection testing will also be used for study testing. If the subject tests positive for *C. difficile*, indicating that their CDI is recurrent and the patient is willing to adhere to a schedule that requires additional stool collections and use of ursodiol for 2 months, the patient will be provided the ursodiol and a kit for the next stool sampling. A copy of the signed consent form will be placed in the subject's research chart to document that informed consent was obtained.

In order to inform physicians, the approved notecards will posted. In addition, the Clinical Microbiology and Immunology laboratory at NYU Langone Medical Center will generate lists of patients for whom *C. difficile* clinical laboratory testing is ordered to help identify potential patients. Furthermore, the study team may also use hospital generated patient censuses of *C. difficile* positive patients to identify potential subjects. We anticipate that most patients identified by the clinical laboratory will be inpatients. We will use their MRNs to identify the location of the patient in the hospital and their treating provider. Patients will be approached in person in the hospital. For relevant outpatients, they will be approached by the preferred phone or email provided in their contact information.

4.4 Early Withdrawal of Subjects

3.41 When and How to Withdraw Subjects

Subjects may withdraw from the study at any time without consequence to their standard treatment. Additionally, subjects will be withdrawn for any of the following reasons:

- The subject ceases to take the ursodiol and/or provide stool samples. If the subject develops symptoms that could be related to CDI a complete evaluation by the principal investigator including fecal bile acid analysis will determine further participation in the study.

A "no show" to a routine visit will prompt a study team member to contact the patient to ascertain current status by reviewing current health and reason for missed visit. The likelihood of recurrence of the diarrhea will be reviewed, but there is no immediate danger to the patient. Should the patient not wish to re-join the study, then it will be suggested that return of diarrhea prompt immediate seeking of medical care.

3.41.1 Data Collection and Follow-up for Withdrawn Subjects

The integrity of the study is not affected by premature withdrawal since early stoppage of ursodiol vitiates the data set which is not included in the final results of the study.

4 Study Drug

4.1 Description:

Ursodiol will be taken as a 300 mg capsule twice a day with or without food. In the unlikely event, based on numbers of patients and years of use, that stools become loose the dose will be lowered to once per day for the reminder of the study. Loose stools are reported in less than 10% of patients on long-term ursodiol therapy.

4.2 Method for Assigning Subjects to Treatment Groups

All *C. difficile* positive patients will be offered the opportunity of co-administration of urso once it is ascertained that they are willing to participate in a two month study with stool collection every two weeks.

4.3 Preparation and Administration of Study Drug

The drug will be purchased by the principal investigator as 300 mg capsules from Lannett Company Inc. that now sells to the NYU outpatient pharmacy. The bottle will be inspected for damaged capsules and a master log sheet containing the manufacturers information as to lot#, shelf life and storage conditions noted.
As patients enter the study, bottles containing 28 capsules will be prepared and labeled ursodiol 300 mg. The study # corresponding to the specimen # and week of the study will be placed on the bottle. It will be given to the study team member who will then give the initial bottle and further bottles at 2 week intervals when a stool specimen is received.

4.4 Subject Compliance Monitoring

The proportion of urso in the fecal bile acid analysis will be the best indication of compliance.
The patient will be informed by the study team if urso is lacking. Causes for non-compliance will be evaluated by the study team, and a decision made as to the likelihood of successful and sustained reentry into study.

4.5 Prior and Concomitant Therapy

Patients entering the study can continue to take all medications that they are currently taking for other existing medical conditions.

4.6 Packaging

Bottles containing twenty eight capsules of ursodiol 300 mg capsules will be given by study physician to patient every two weeks.

4.7 Receiving, Storage, Dispensing and Return

4.7.1 Receipt of Drug Supplies

Medications will be dispensed by the NYU pharmacy, either to the patient directly or to a study team member who will then provide the medication to the patient. Patients hospitalized at Bellevue will receive the study medication through the Bellevue Research Pharmacy as a non-formular research drug.The bottle will be inspected for damage and a drug receipt log initiated

4.7.2 Storage

The drug will be stored in accordance with the manufacturers recommendation. It has a shelf life of 1 year at 25 C and does not require protection from light. The principal investigator will monitor and manage the storage of the drug.

4.7.3 Dispensing of Study Drug

At the beginning of the study, once informed consent has been obtained, the subject will receive a bottle containing 28 capsules of 300 mg ursodiol with instruction to take a capsule in the morning and evening. If subjects taking bile sequestrant drugs are enrolled, they will be instructed to take ursodiol at least 5 hours before or after they take the bile acid sequestrant.

When the subject returns to the study-team in 14 days, the bottle will be brought back and a new bottle containing 28 capsules given to the subject. We have no precise way of knowing, other than the patient's integrity, how much of the medication is actually taken (skipped doses) but analysis of the fecal specimen for urso will give an approximate indication of compliance. The study team will note any capsules remaining in the bottle at each visit.

For each follow up visit, a -4 day window will be used to account for potential scheduling difficulties and limit the chances of the patient running out of medication. The patient will have at most 8 pills when returning for their follow-up visit. During this follow-up visit, the patient will take back their previously dispensed bottle, as well as a newly dispensed bottle, to take home. The patient will be instructed to continue their previously dispensed bottle of ursodiol until they are finished with the original bottle. Afterwards, patients can then start their newer bottle. The patients will be instructed to bring all medication bottles, whether empty or with remaining ursodiol.

The patients must bring a stool sample when obtaining the next bottle of medicine and for the last follow-up visit. Patients are given no more than 28 pills per dispensation to ensure patients will come back with a stool sample and to obtain the next bottle of pills.

For patients at Bellevue, who are identified while hospitalized, study drug will be dispensed by the Bellevue Research Pharmacy. Upon discharge, patient will be provided outpatient medication as described above.

4.7.4 Return or Destruction of Study Drug

At the completion of the study, there will be a final reconciliation of drug shipped, drug consumed, and drug remaining by the Principal Investigator. This reconciliation will be logged on the drug reconciliation form, signed and dated. Any discrepancies noted will be investigated, resolved, and documented prior to return or destruction of unused study drug. Drug destroyed on site will be documented in the study files.

5 Study Procedures

5.1 Visit 1. Presumptionof C. difficile colitis & diarrhea is established.

- Study is explained to patient, informed consent obtained
- Review of medical history, physical exam, height, weight, and vital signs recorded from clinical/standard care follow-up visit with physician
- Subjects instructed to collect a stool sample for *C. difficile* testing and/or study testing.
- Subjects are provided with a study survey.
- *C. difficile* negative patients are finished with their study participation.
- *C. difficile* positive patients are given ursodiol and antibiotics.
- Potential adverse events are reviewed with the *C. difficile* positive patients.

5.2 Visit 2. Two weeks later. (C. difficile positive patients)

- Antibiotic are likely to have been discontinued
- Stool specimen and study survey obtained.
- 28 Capsules of 300mg urso dispensed with instructions of 1 capsule twice a day.
- Adverse events follow-up

5.3 Visit 3. Two weeks later. (C. difficile positive patients)

- Stool specimen and study survey obtained.
- Dispense 28 capsules urso 300 mg (collect bottle from previous visit)
- Adverse events follow-up

5.4 Visit 4. Two weeks later. (C. difficile positive patients)

- Stool specimen and study survey obtained.
- dispense 28 capsules urso 300 mg (collect bottle from previous visit)
- Adverse events follow-up

5.5 Visit 5: Two weeks later. (End of study drug administration) (C. difficile positive patients)

- Stool specimen and study survey obtained.
- Collect unused bottle from previous visit
- Adverse events follow-up

The table below shows each visit and the procedures done at each visit:

Activity	**Visit Name** #1	**Visit Name** #2[b]	**Visit Name** #3[b]	**Visit Name** #4[b]	**Visit Name #5[b]**	**Visit Name #6 (Optional)**	**Visit Name #7 (Optional)**
Study team procedures							
Consent	X						
Medical History	X						
Study drug dispensation (ursodiol)	x	X	x	x			
Study health and dietary survey	x	x	x	x	x	x	x
Adverse Event review	x	x	x	x	x		

Laboratory Assessments							
C. difficile	**Positive (continue visits 2-5)**						
	negative						
Stool collection	***x***[a]	***x***	***x***	***x***	***x***	***x***	***x***

a. baseline stool sample can be collected on site if possible or before medication therapy is initiated
b. Each follow-up visit will have a -4 day window to account for potential scheduling difficulties. Patients will have at most 8 pills when returning for follow-up visit

6 Statistical Plan

6.1 Sample Size Determination is based on (1) the likelihood of obtaining

Up to 32 *C. difficile* positive patients will be enrolled so that we will reach a goal of 30 subjects who complete the study within a 10 month period taking screen-failures/dropouts into consideration. The recurrence rate of *C. diffiicle* in patients that were given either metronidazole or vancomycin is estimated at 25% and several patients a month are treated both at Bellevue and at Tisch for recurrent infection. Estimating that 4 patients a month will participate in the study will yield 32 patients in 8 months and completion of the study in 10 months.

Assuming the correctness of the report that ursodeoxycholic acid (figure 2) suppresses *C. difficle* growth then the major question we wish to answer is the frequency of return to a normal fecal bile acid pattern during the two month period.

We will enroll both *C. difficile* positive and *C. difficile* negative patients until the goal of 32 *C. difficile* positive patients is reached.

6.2 Statistical Methods: We will be analyzing for the following fecal bile acids

(1) Taurine conjugates (2) glycine conjugates (3) non conjugated (a) cholic acid (b) chenodeoxycholic acid (c) deoxycholic acid (d) ursodeoxycholic acid (e) lithocholic acid, each expressed as a per cent of the total.

Based on studies in the literature, the initial fecal sample is expected to have an increase in the proportion of conjugated bile acids and non-conjugated cholic and chenodeoxycholic acid with absent deoxycholic, lithocholic and ursodeoxycholic acid.

Subsequent stool specimens are all expected to contain ursodeoxycholic acid and therefore a reduction in the proportion of conjugated bile acids. The “key” data will be the increase in the proportion of deoxycholic and lithocholic acid and decrease in chenodeoxycholic and cholic acids which will **only occur with the return of the normal enteric bacterial flora** (confirmed via microbiome analyses).

The percent of patients with return toward a normal fecal bile acid pattern will be the criterion for evaluating the success of urso treatment. If all subjects remain symptom-free during the study then urso may be considered as a surrogate for FMT. Return of the endogenous deoxycholic acid pool in 20% or more of subjects would indicate they probably will not need FMT therapy and are protected from recurrent CDI.

6.3 Subject Population(s) for Analysis

In addition to the *C. difficile* negative stool specimens, only subjects that have completed the two month period and provided 5 stool samples will be included in the data analysis. *C. difficile* negative stool samples and initial fecal samples from *C. difficile* positive subjects that did not complete the study will be used to compare to literature reports on the fecal bile acid patterns of individuals with recurrent *C. difficile* infection.

7 Safety and Adverse Event

Adverse Event

Based on published experience with the use of ursodeoxycholic acid, it is possible that some patients may develop loose bowel movements at a total dose of 600 mg/day. In this event, the daily dose will be lowered to 300 mg/day and the subject remain in the study.

Serious Adverse Event
The recurrence of symptoms together with evidence of recurrent *C. difficile* infection during the two month period would indicate the emergence of a resistant strain of bacterium that will require medical care beyond the scope of the study. However, such an event will be included in the final published report.

7.1 Recording of Adverse Events

At each contact with the subject, the study team member must seek information on adverse events by specific questioning and, as appropriate, by examination. Information on all adverse events should be recorded immediately in the source document, and also in the appropriate adverse event module of the case report form (CRF). All clearly related signs, symptoms, and abnormal diagnostic procedures results should recorded in the source document, though should be grouped under one diagnosis.

All adverse events occurring during the study period must be recorded. The clinical course of each event should be followed by the study team until resolution, stabilization, or until it has been determined that the study treatment or participation is not the cause. Serious adverse events that are still ongoing at the end of the study period must be followed up to determine the final outcome. Any serious adverse event that occurs after the study period and is considered to be possibly related to the study treatment or study participation should be recorded and reported immediately.

7.2 Reporting of Serious Adverse Events and Unanticipated Problems

The sponsor-investigator must conform to the adverse event reporting timelines, formats and requirements of the various entities to which they are responsible, but at a minimum those events that must be reported are those that are:

- related to study participation,
- unexpected, and
- serious or involve risks to subjects or others
  (see definitions, section 7.1).

For Narrative Reports of Safety Events
If the report is supplied as a narrative, the minimum necessary information to be provided at the time of the initial report includes:

- Study identifier
- Study Center
- Subject number
- A description of the event
- Date of onset
- Current status
- Whether study treatment was discontinued
- The reason why the event is classified as serious
- Investigator assessment of the association between the event and study treatment

7.2.1 Investigator reporting: notifying the sponsor-investigator (Principal Investigator)

The following describes events that must be reported to the sponsor-investigator in an expedited fashion.

Initial Report: within 24 hours:
The following events must be reported to the study sponsor by telephone within 24 hours of awareness of the event: recurrence of symptoms to be evaluated by the physician lowering dose of urso

- Unanticipated problems related to study participation, loss of urso medication
- Serious adverse events, recurrence of *C. difficile* infection

Follow-up report: within 48 hours:
As a follow-up to the initial report, within the following 48 hours of awareness of the event, the investigator shall provide further information, as applicable, on the unanticipated device event or the unanticipated problem in the form of a written narrative. This should include a copy of the completed Unanticipated Problem form, and any other diagnostic information that will assist the understanding of the event. Significant new information on ongoing unanticipated adverse device effects shall be provided promptly to the sponsor-investigator.

Other Reportable events:

- **Deviations from the study protocol**
  Deviations from the protocol must receive both sponsor-investigator and IRB approval before they are initiated. Any protocol deviations initiated without sponsor-investigator and IRB approval that may affect the scientific soundness of the study, or affect the rights, safety, or welfare of study subjects, must be reported to the sponsor-investigator and IRB as soon as a possible, but ***no later than 5 working days*** of the protocol deviation.

- ***Withdrawal of IRB approval***
  An investigator shall report to the sponsor a withdrawal of approval by the investigator's reviewing IRB as soon as a possible, but ***no later than 5 working days*** of the IRB notification of withdrawal of approval.

7.2.2 Sponsor-Investigator reporting: notifying the IRB

Federal regulations require timely reporting by investigators to their local IRB of unanticipated problems posing risks to subjects or others. The following describes the NYULMC IRB reporting requirements, though Investigators at participating sites are responsible for meeting the specific requirements of their IRB of record.

Report Promptly, but no later than 5 working days:
Researchers are required to submit reports of the following problems promptly but no later than 5 working days from the time the investigator becomes aware of the event:

- ***Unanticipated problems including adverse events*** **that are unexpected and related**

- *Unexpected: An event is "unexpected" when its specificity and severity are not accurately reflected in the protocol-related documents, such as the IRB-approved research protocol, any applicable investigator brochure, and the current IRB-approved informed consent document and other relevant sources of information, such as product labeling and package inserts.*
- *Related to the research procedures: An event is related to the research procedures if in the opinion of the principal investigator or sponsor, the event was more likely than not to be caused by the research procedures.*
- *Harmful: either caused harm to subjects or others, or placed them at increased risk*

Other Reportable events:
The following events also require prompt reporting to the IRB, though ***no later than 5 working days***:

- ***Complaint of a research subject*** when the complaint indicates unexpected risks or the complaint cannot be resolved by the research team.
- ***Protocol deviations or violations*** (includes intentional and accidental/unintentional deviations from the IRB approved protocol) for any of the following situations:
  - *one or more participants were placed at increased risk of harm*
  - *the event has the potential to occur again*
  - *the deviation was necessary to protect a subject from immediate harm*
- ***Breach of confidentiality***
- ***Incarceration of a participant*** when the research was not previously approved under Subpart C and the investigator believes it is in the best interest of the subject to remain on the study.
- ***New Information indicating a change to the risks or potential benefits*** of the research, in terms of severity or frequency. (e.g. analysis indicates lower-than-expected response rate or a more severe or frequent side effect; Other research finds arm of study has no therapeutic value; FDA labeling change or withdrawal from market)

Reporting Process
The reportable events noted above will be reported to the IRB via: "Reportable New Information" submission or as a written report of the event (including a description of the event with information regarding its fulfillment of the above criteria, follow-up/resolution and need for revision to consent form and/or other study documentation).

Copies of each report and documentation of IRB notification and receipt will be kept in the Clinical Investigator's study file.

7.3 Data Safety Monitoring Plan

It is the responsibility of the Principal Investigator to oversee the safety of the study at his/her site. This safety monitoring will include careful assessment and appropriate reporting of adverse events as noted above, as well as the construction and implementation of a site data and safety-monitoring plan as follows: Dr. Michael Poles, M.D., Ph.D. , Associate Professor of Medicine, Microbiology and Immunology at NYU School of Medicine who is a Gastroenterologist and conducted his own research programs will review the fecal bile acid data and ask providers about the recurrence of symptoms in subjects at two week intervals. Recurrence of symptoms that are confirmed to be attributable to CDI in a subject with ursodeoxycholic acid in the stool sample will be an indication for withdrawing from the study. Recurrence of symptoms confirmed to be related to CDI and failure to find increasing amounts of deoxycholic acid in the initial 4 subjects would be an indication for suspending, modifying or stopping the study. Summarized reports of these reviews will be submitted

to the IRB after 10 subjects have completed the study. Additional summarized reports will be submitted after 20 subjects have completed the study, and after all subjects have completed the study. Other reports will be submitted if untoward events occur and/or the monitor determines the study needs to be suspended, modified, or stopped.

7.4 Confidentiality

The only research data collected will be fecal bile acid analysis which will be entered on Excel data sheets with study numbers instead of identifiers. Only study team members will be able to relate the study number to a specific patient. All data will be stored on password protected computers within the NYU medical center secure network and de-identified data sheets sent only to physicians listed in the study with e-mail addresses at nyumc.org.

For stool specimens collected during the study, the specimen ID (subject # and specimen collection # i.e. 1234-F1, 1234-F2, etc.) will be used to identify the sample and no personal identifiers will be used.

7.5 Confidentiality and HIPAA

Information about study subjects will be kept confidential and managed according to the requirements of the Health Insurance Portability and Accountability Act of 1996 (HIPAA). Those regulations require a signed subject authorization informing the subject of the following:

- What protected health information (PHI) will be collected from subjects in this study
- Who will have access to that information and why
- Who will use or disclose that information
- The rights of a research subject to revoke their authorization for use of their PHI.

In the event that a subject revokes authorization to collect or use PHI, the investigator, by regulation, retains the ability to use all information collected prior to the revocation of subject authorization. For subjects that have revoked authorization to collect or use PHI, attempts should be made to obtain permission to collect at least vital status (i.e. that the subject is alive) at the end of their scheduled study period.

7.6 Source Documents

Source data is all information, original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Source data are contained in source documents. Examples of these original documents, and data records include: hospital records, clinical and office charts, laboratory notes, memoranda, subjects' diaries 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, subject files, and records kept at the pharmacy, at the laboratories, and at medico-technical departments involved in the clinical trial.

7.7 Case Report Forms

The study case report form (CRF) is the primary data collection instrument for the study. All data requested on the CRF must be recorded. All missing data must be explained. If a space on the CRF is left blank because the procedure was not done or the question was not asked, write "N/D". If the item is not applicable to the individual case, write "N/A". All entries should be printed legibly in black ink. If any entry error has been made, to correct such an error, draw a single straight line through the incorrect entry and enter the correct data above it. All such changes must be initialed and dated. DO NOT ERASE OR WHITE OUT ERRORS. For clarification of illegible or uncertain entries, print the clarification above the item, then initial and date it.

7.8 Records Retention

It is the investigator's responsibility to retain study essential documents for at least 2 years after the last approval of a marketing application in their country and until there are no pending or contemplated marketing applications in their country or at least 2 years have elapsed since the formal discontinuation of clinical development of the investigational product. These documents should be retained for a longer period if required by an agreement with the sponsor. In such an instance, it is the responsibility of the sponsor to inform the investigator/institution as to when these documents no longer need to be retained.

8 Study Monitoring, Auditing, and Inspecting

8.1 Study Monitoring Plan

This study will be monitored according to the following monitoring plan: Dr. Michael Poles, the data safety monitor will ensure regulatory compliance and proper protocol conduct at 2 week intervals.
The investigator will allocate adequate time for such monitoring activities. The Investigator will also ensure that the monitor or other compliance or quality assurance reviewer is given access to all the above noted study-related documents and study related facilities (e.g. pharmacy, diagnostic laboratory, etc.), and has adequate space to conduct the monitoring visit.

8.2 Auditing and Inspecting

The investigator will permit study-related monitoring, audits, and inspections by the IRB/EC, government regulatory bodies, and University compliance and quality assurance groups of all study related documents (e.g. source documents, regulatory documents, data collection instruments, study data etc.). The investigator will ensure the capability for inspections of applicable study-related facilities (e.g. pharmacy, diagnostic laboratory, etc.).

Participation as an investigator in this study implies acceptance of potential inspection by government regulatory authorities and applicable University compliance and quality assurance offices.

9 Ethical Considerations

This study is to be conducted accordance with applicable US government regulations and international standards of Good Clinical Practice, and applicable institutional research policies and procedures.

This protocol and any amendments will be submitted to a properly constituted Institutional Review Board (IRB)) in agreement with local legal prescriptions, for formal approval of the study conduct. The decision of the IRB concerning the conduct of the study will be made in writing to the investigator and a copy of this decision will be provided to the sponsor-investigator before commencement of this study along with a list of IRB members.

All subjects for this study will be provided a consent form describing this study and providing sufficient information for subjects to make an informed decision about their participation in this study. This consent form will be submitted with the protocol for review and approval by the IRB for the study. The formal consent of a subject, using the IRB -approved consent form, must be obtained before that subject undergoes any study procedure. The consent form must be signed by the subject and the investigator-designated research professional obtaining the consent.

10 Study Finances

10.1 Funding Source

This study will be funded by the Division of Gastroenterology using funds designated for research purposes.

10.2 Conflict of Interest

Any study team member who has a conflict of interest with this study (patent ownership, royalties, or financial gain greater than the minimum allowable by their institution, etc.) must have the conflict reviewed by a properly constituted Conflict of Interest Committee with a Committee-sanctioned conflict management plan that has been reviewed and approved by the study sponsor prior to participation in this study. All NYULMC study team personnel will follow the applicable University conflict of interest policies.

10.3 Subject Stipends or Payments:

There are no subject stipend/payments. Ursodiol will be provided without cost. Subjects and/or their insurance plans will not be billed for study-related stool samples.

11 Publication Plan

Neither the complete nor any part of the results of the study carried out under this protocol, nor any of the information provided by the sponsor for the purposes of performing the study, will be published or passed on to any third party without the consent of the principal investigator. Any study team member involved with this study is obligated to provide the principal investigator with complete test results and all data derived from the study. The principal investigator will prepare a manuscript for and circulate it for comments and approval to all study participants prior to submission for publication

12 References

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