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Fundamental Modification of the Gut Microbiota in the Treatment of Refractory Crohn's Disease

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Funding Sponsor: The Broad Medical Research Program at CCFA

Investigational Product: Neomycin, vancomycin, ciprofloxacin, fluconazole, polyethylene glycol 3350, promethazine

IRB Number: 823635

IND/IDE Number: IND Exemption granted by Penn Office of Clinical Research

Initial version: 2015-10-13 v1.0
Amended: **v2.0.20160502**
Amended: **v3.0.20160623**
Amended: **v4.0.20160729**
Amended: **v5.0.20160810**
Amended: **v6.0.20160920**
Amended: **v7.0.20170210**
Amended: **v8.0.20170313**
Amended: **v9.0.20170512**
Amended: **v10.0.20170811**

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List of Abbreviations

AE	Adverse event
CBC	Complete blood count
CD	Crohn's disease
CDAI	Crohn's Disease Activity Index
CHOP	The Children's Hospital of Philadelphia
CMP	Comprehensive metabolic panel
CMV	Cytomegalovirus
CRF	Case Report Form
CRP	C-reactive protein
CTRC	Clinical and Translational Research Center
EKG	Electrocardiogram
ESR	Erythrocyte sedimentation rate
FCP	Fecal calprotectin
GI	Gastrointestinal
HBI	Harvey Bradshaw Index
hsCRP	High-sensitivity CRP
IBD	Inflammatory bowel disease
IBDU	Indeterminate colitis
ICE	Idiopathic chronic enterocolitis
IDS	Investigational Drug Service
IRB	Institutional Review Board
ITS	Internal Transcribed Spacer
LOR	Loss of responsiveness
MRN	Medical record number
PEG	Polyethylene glycol
PENN	University of Pennsylvania
PCDAI	Pediatric Crohn's Disease Activity Index
PUCAI	Pediatric Ulcerative Colitis Activity Index
SAE	Serious adverse event
sCDAI	Short CDAI
TUNPRC	Tulane University National Primate Center
UC	Ulcerative colitis

Study Summary

Title	Fundamental Modification of the Gut Microbiota in the Treatment of Refractory Crohn's Disease
Short Title	Holiday
IRB Number	823635
Protocol Number	N/A
Phase	Phase II
Methodology	Randomized, placebo-controlled, double blind
Study Duration	Individual participant participation will last 6-7 months; full study procedures are expected to last 2 years.
Study Center(s)	University of Pennsylvania
Objectives	**Primary Objective:** • To determine the effect of a novel gut microbiota-targeted therapeutic regimen (bowel lavage and antibiotics with or without an antifungal) in the management of active CD that is refractory to conventional, immunosuppressive therapy. **Secondary Objective(s):** • To correlate the effectiveness in reducing bacterial 16S rRNA copy number and fungal 18S rRNA copy number, with the use of the regimen in the primary objective, with improvement of disease activity in patients with CD refractory to immunosuppressive therapy. • To determine the relationship between the effectiveness of the proposed therapies with changes in gut microbiota composition.
Number of Participants	30 participants will be enrolled with an estimated yield of 20 evaluable participants.

Main Inclusion and Exclusion Criteria	**Inclusion Criteria:** • Participants ages 18-75 • Active moderate CD defined as Harvey Bradshaw Index (HBI) ≥ 7 • Have had primary nonresponse or an initial response for 8 or more weeks, followed by loss of responsiveness (LOR) (self-reported worsening of symptoms for at least 7 days), to one or more of the following therapies*: azathioprine, 6-mercaptopurine, methotrexate, adalimumab, certolizumab, golimumab, infliximab, natalizumab, vedolizumab, or ustekinumab *Must be administered at standard, therapeutic dosages **Exclusion Criteria:** • Known or suspected stricturing disease producing obstructive symptoms • Known allergy or intolerance to any of the medications used in this study • Elevated creatinine • Prolonged QTc interval as seen on enrollment EKG
Investigational Product (drug, dose, route, regimen)	Vancomycin 500 mg suspension four times daily (Day 1-14), plus neomycin 1000 mg orally three times daily (Days 1-3), plus ciprofloxacin 750 mg orally twice daily (Day 4-14), plus Polyethylene Glycol 3350 (Miralax) 238 g dissolved in 64 ounces of Gatorade or Crystal Lite on day 2, plus fluconazole 400 mg orally once daily (Day 1-14) or placebo. PRN Promethazine 12.5mg up to every four hours (Days 1-3).
Duration of administration	14 days
Reference therapy	N/A
Statistical Methodology	Our estimates of efficacy will use a disease activity score (HBI) for clinical response, FCP for mucosal inflammation, and hsCRP for systemic inflammation. The two treatment groups will be compared using standard descriptive statistics. Categorical variables will be compared using Fisher's exact test and continuous variables will be compared using the unpaired t-test or Wilcoxon rank sum test if the data are not normally distributed.
Safety Evaluations	Primary measurements that will be used to assess safety include vital signs, laboratory results, EKG for QTc interval, disease activity scores, and screening for medication side effects and adverse events.
Data and Safety Monitoring Plan	Monitoring is risk based.

BACKGROUND AND STUDY RATIONALE

This study will be conducted in full accordance with all applicable University of Pennsylvania Research Policies and Procedures and all applicable Federal and state laws and regulations including 45 CFR 46, 21 CFR Parts 50, 54, 56, and Good Clinical Practice: Consolidated Guidelines approved by the International Conference on Harmonization (ICH). All episodes of noncompliance will be documented.

This document is a clinical research protocol and the investigators will perform the study in accordance with this protocol, will obtain consent, and will report unanticipated problems involving risks to participants or others in compliance with the University of Pennsylvania Research Policies and Procedures and all federal requirements. Collection, recording, and reporting of data will be accurate and will ensure the privacy, health, and welfare of research participants during and after the study.

Introduction

Recent evidence suggesting that the commensal gut microbiota, or the "gut microbiome," is responsible for stimulation of the intestinal immune system in inflammatory bowel disease (IBD) has caused many researchers and providers in the field to question current therapeutic approaches. Though most IBD providers would agree that immunosuppressive medications are *currently* the most reliable therapies, such research indicates that if gut flora plays a role in the pathogenesis of IBD, then perhaps targeting commensal microbes rather than or in addition to the immune system would be more efficacious.

This protocol seeks to test the hypothesis that a therapeutic approach that deeply alters the composition and bacterial/fungal load of the gut microbiota will be efficacious in patients with refractory Crohn's Disease (CD) who have primary nonresponse or secondary loss of response to conventional, immune suppressive therapies. This study will evaluate the efficacy of a novel treatment regimen employing non-immunosuppressive medications in the management of refractory CD. Patients will be treated with a combination of gut microbiota-targeted therapies to deeply modify the dysbiotic gut microbiota that has been consistently reported in patients with CD[1]. We believe this strategy will both treat the gut inflammation associated with IBD, as well as rescue response to biologic or immunomodulator therapies.

1.1 Background and Relevant Literature

1.1.1 Inflammatory Bowel Disease: Current Therapies and Treatment Challenges

Inflammatory bowel disease (IBD), including CD and UC, affects approximately 1.5 million Americans and the incidence is increasing worldwide [2]. Current evidence indicates that the pathogenesis of IBD involves an inappropriate and persistent inflammatory response to the gut microbiota in genetically susceptible individuals [3]. In support of this notion, several studies utilizing animal models have shown that the development of intestinal inflammation requires microbial colonization of the gut [4, 5]. Clinical observations of IBD further implicate the role of the commensal gut microbiota, as IBD usually affects intestinal regions with the highest bacterial load, and both fecal diversion and antibiotic treatment can be effective in the management of CD [6, 7].

However, standard treatments for IBD do not focus on restoring immune tolerance to commensal microbes but rather depend on immunosuppression. Although effective in inducing and maintaining remission for many patients, there is substantial risk of side effects associated with the use of immunosuppressive medications, namely steroids, immunomodulators (e.g., thiopurines and methotrexate) and biologics (e.g., infliximab and adalimumab) [8]. In addition, a significant proportion of patients will experience primary nonresponse or a loss of response (LOR) to immunosuppression over time in a way that cannot be explained by the pharmacokinetics of the drug [9].

When nonresponse or LOR occurs, providers must escalate dosage or change the treatment medication(s). Because of the limited number of medications and concern for the exhaustion of non-

surgical options, maintaining patients with IBD on an effective medication for as long as possible becomes a priority of clinical care.

1.1.2 History of Gut Microbiota-Targeted Therapies for IBD

Antibiotics

There is clear evidence for the effectiveness of antibiotics in the treatment of inflammation in animal models of IBD [10-12]. For example, IL-10 knockout mice develop a phenotype comparable to human IBD and at least two studies have shown that antibiotics such as neomycin, ciprofloxacin, vancomycin, and metronidazole may both prevent and treat intestinal inflammation [10, 12]. However, the evidence for the effectiveness of antibiotics in the treatment of humans with IBD has historically been less robust. Within the past several years, two meta-analyses of randomized controlled trials have documented a small but statistically significant benefit of antibiotics to induce remission in both CD and UC [13, 14]. In addition, several studies have now shown that antibiotic combination therapy significantly improves rates of remission and also steroid withdrawal in UC [15-18].

Most notably, Turner and colleagues have recently published their experience using a two- to three-week course of combination antibiotic therapy in pediatric UC and indeterminate colitis refractory to standard immunosuppressive medications [18]. Patients were treated with combination oral amoxicillin, metronidazole, and doxycycline, except in children 2-7 years old where doxycycline was substituted with ciprofloxacin, and in infants under 2 years old where doxycycline was substituted with gentamicin. In addition, in cases of allergy the allergenic drug was substituted with gentamicin, and in hospitalized children vancomycin was added to the regimen. The antibiotic regimen was definitively effective in 7/15 (47%) of patients, inducing complete clinical remission as defined by the Pediatric Ulcerative Colitis Activity Index (PUCAI). For the patients defined as primary responders (n=9), i.e., clinical remission at three weeks after initiating antibiotic therapy, reduction in CRP and PUCAI were statistically significant. Finally, all patients repeatedly tested negative for fecal *C. difficile* and bacterial cultures indicative of developed resistance. Only one patient tested positive for CMV and was subsequently treated with ganciclovir. These findings demonstrate the potential efficacy of an antibiotic therapeutic strategy in the treatment of refractory IBD.

Antifungals

In addition to the bacterial component, there appears to be a relationship between IBD and the fungal gut microbiota. The authors of a recent study demonstrated that mice lacking Dectin-1, a C-type leptin receptor that recognizes β-glucans in the fungal cell wall, had increased susceptibility to chemically induced colitis due to their altered immunological responses to indigenous fungi [19]. Significantly, a polymorphism in the gene encoding Dectin-1 (CLEC7A) was found to be associated with a severe form of ulcerative colitis in humans [19]. A connection between high dietary concentrations of yeast and increased disease activity in patients with CD has also been suggested [20].

In terms of fungal-targeted therapies for IBD, preliminary evidence suggests that fluconazole treatment may reduce intestinal inflammation in animal models of colitis and in patients with IBD [21]. We recently demonstrated a significant difference in the composition of the fungal microbiota in pediatric patients with IBD as compared to healthy controls [2], further suggesting a relationship between fungi and the pathogenesis of IBD.

Bowel Lavage

Given the potential role of the gut microbiota in the pathogenesis of IBD, therapies that deplete commensal flora in the gastrointestinal (GI) tract have been suggested. For example, in the 1980's, intestinal lavage with normal saline was studied in patients hospitalized with acute exacerbations of severe CD. A small, controlled study demonstrated reduction in disease severity and also duration of hospitalization [23, 24]. Failure to further develop bowel lavage as an independent therapeutic option is

likely secondary to advancements in pharmacological therapies. Recent evidence has indeed shown that bacterial diversity in the gut is significantly decreased following colonoscopy preparations [25].

1.1.3 Preliminary Data

Effectiveness of Antibiotics in Mouse Models versus Humans, What's the Difference?

The observation that antibiotics as currently used have only modest efficacy in the treatment of CD and UC represents a challenge to the notion that antimicrobials could be used to deplete the microbiota in patients with IBD. One possible explanation is that the use of antibiotics in animal models is more effective in reducing intestinal bacterial load in mice than in humans. Indeed, in preliminary data [3], we show that two specific oral antibiotics dramatically reduce bacterial load by greater than 4 logs in mice within 72 hours, as quantified by 16S gene copy number.

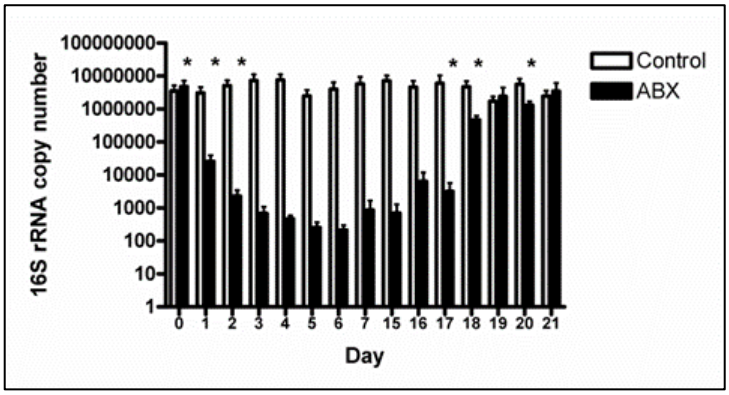

Figure 2: Time course of 16S rRNA gene copy number during oral antibiotic treatment (14 days of vancomycin plus neomycin) and upon discontinuing antibiotics on day 15 compared to control.

We believe that an antibiotic therapeutic strategy capable of significantly reducing bacterial load in patients with CD will show greater levels of efficacy in the treatment of active disease than in previously reported studies [26-28]. Additionally, based on data suggesting the potential importance of gut fungi in IBD, we hypothesize that to focus exclusively on gut bacterial load may represent a missed opportunity.

Efficacy of the Holiday Regimen for Treatment of Chronic Enterocolitis in Rhesus Macaques (Macaca mulatta)

Idiopathic chronic enterocolitis (ICE) is one of the most significant causes of morbidity and mortality in captive nonhuman primates and remains a diagnostic and therapeutic challenge for veterinarians working with these species. Current evidence suggests that ICE is a multifactorial disease involving perturbations in gastrointestinal bacterial populations, as well as the response of the host immune system to these changes [29]. Our group is currently collaborating with Tulane University National Primate Center (TUNPRC) on a study where rhesus macaques with ICE and a negative stool infectious work-up (n=6 at present) were treated with an anti-microbial regimen similar to the one proposed here. The animals receive the following regimen: 125 mg total vancomycin hydrochloride four times daily, 50 mg/kg neomycin twice daily, and fluconazole 2 mg/kg twice daily. Therapy was administered for a total of 14 days. Thus far, four of the six animals have experienced no signs of diarrhea since completion of the treatment protocol. Soft stool was observed for one day in the remaining two monkeys since completion of treatment, with normal stool observed on other days for both animals. Based upon daily observations of mentation/behavior, activity level, and appetite as well as pre-treatment and intra-treatment CBC and serum chemistries, this treatment regimen was well-tolerated by all animals with no adverse effects noted.

Efficacy of Combination Antibiotic Therapy for Refractory IBD at CHOP

Since the paper by Turner and colleagues [18] was presented at a national meeting in 2014, physicians within the CHOP Center for Inflammatory Bowel Disease have been utilizing a combination antibiotic approach clinically for patients with IBD refractory to standard therapy. As a means of preliminary efficacy evaluation, we performed a retrospective study (CHOP IRB #15-011806) to review our experience treating refractory IBD with combination antibiotic therapy. Information collected included patient demographics, disease characteristics, immunotherapy history, indication for antibiotic therapy, and type, dosage, and duration of antibiotics prescribed. Eligible patients were ages 3-21 and prescribed treatment with three or more oral antibiotics concomitantly for the treatment of IBD. Clinical outcomes were evaluated based on changes in disease activity, as measured by the Pediatric Crohn's Disease Activity Index (PCDAI) in participants with CD, or the Pediatric Ulcerative Colitis Activity Index (PUCAI) in patients with UC or indeterminate colitis, at the time of initiation of combination antibiotic therapy and at multiple subsequent time points. Disease morbidity outcomes were additionally measured, including referral to and/or scheduling of surgery, as well as escalation of therapy to an experimental medication. The incidence of adverse reactions during and following combination antibiotic therapy was also assessed.

Of the enrolled patients (n=14), four participants had CD (29%) and the remainder had UC or indeterminate colitis (IBDU) (Table 1). In 13 participants, the indication for combination antibiotic therapy was disease refractory to standard immunosuppressive therapies and in the remaining participant the indication for antibiotic therapy was induction and maintenance of remission. Participants were prescribed combination antibiotic therapy for an average of 29.5 days (range 9-71).

TABLE 1: PEDIATRIC IBD PATIENTS TREATED AT CHOP WITH COMBINATION ANTIBIOTICS

Case Number	Age[a]	IBD Type	LOR to Medications[b]	Antibiotic Regimen[c]	Duration in Days	Surgery Referral	Experimental Therapy[d]	Rescue of Response
1	13.2	UC	IFX+MTX	MAC	14	Cancelled	No escalation	IFX+MTX
2	12.3	UC	IFX	MADV	71	Delayed (96 days)	n/a	IFX
3	16.4	CD	IFX+MTX, ADA+MTX	MCV	23	n/a	No escalation	ADA+MTX
4	10.8	CD	IFX+MTX	MAC	9	Proceeded	n/a	n/a
5	13.1	IBDU	IFX, ADA+MTX	MAC	24	Cancelled	n/a	ADA+MTX
6	15.7	CD	IFX+MTX	MACV	28	n/a	Escalation	n/a
7	13.6	UC	IFX+MTX	MAC	21	n/a	n/a	n/a
8	18.5	UC	IFX+MTX	MAC	59	Cancelled	No escalation	n/a
9	11.5	UC	IFX	RCV	35	n/a	n/a	IFX+MTX
10	10.7	IBDU	IFX+MTX, ADA	MCV	19	Delayed (22 days)	No escalation	n/a
11	6.5	CD	IFX+MTX, ADA+MTX	MCV	36	n/a	n/a	ADA+MTX
12	16.1	CD	IFX	MCDV	31	n/a	No escalation	IFX+MTX
13	12.3	UC	n/a	MCV	21	n/a	n/a	n/a
14	10.4	UC	IFX	MCDV	22	Cancelled	n/a	n/a

[a]Age at initiation of antibiotic therapy

[b]All demonstrated LOR; IFX=infliximab monotherapy, IFX+MTX=IFX plus methotrexate dual therapy, ADA+MTX=adalimumab plus methotrexate dual therapy

[c]A=amoxicillin, C=ciprofloxacin, D=doxycycline, M=metronidazole, R=rifaximin, V=vancomycin

[d]Referral or discussion of eligibility screening for vedolizumab (Entyvio) or ustekinumab (Stelara)

Because of the limitations inherent to the review of existing medical records, disease activity scores at all study time points were not available for each participant. However, for those participants with available

data, PUCAI and PCDAI scores showed decreases in disease activity both two weeks post-initiation and post-completion of their prescribed antibiotic combination regimen.

There were four documented adverse reactions that occurred during the course of therapy or shortly following therapy termination. All were mild and only one was probably related to the medication regimen (black, hairy tongue); all resolved without sequelae. There were two severe and one life-threatening adverse event; however, all occurred two weeks after termination of therapy and based on clinical expertise were unlikely related to the antibiotic therapy. Only one additional event was possibly related to the prescribed antibiotic therapy – a vaginal yeast infection that occurred four weeks post-termination of therapy and which also resolved without sequelae.

This proposal will test the hypothesis that the gut microbiota is fundamentally involved in the perpetuation of CD that is refractory to conventional strategies and that a strategy that dramatically alters the composition and/or biomass of the gut microbiota (both bacteria and fungi) will lead to clinical improvement and reduction of inflammation.

1.2 Investigational Regimen and Rationale

In this protocol, we will allow the gastrointestinal tract a "Holiday" from gut microbes that may be perpetuating the inflammatory response. We will attempt to reduce the microbial load in the gut through an intestinal lavage followed by short-term (14 days) treatment with broad-spectrum antibiotics with or without an antifungal. The intestinal lavage protocol was chosen based on the standard bowel cleanse protocol utilized at The University of Pennsylvania hospitals prior to colonoscopy and are also widely accepted in the literature [30]. In our animal study above (Figure 1), we were able to significantly reduce 16S rRNA gene copy number with a combination of two oral antibiotics – neomycin and vancomycin. In this protocol, we have adapted the regimen for human use because of the risk of ototoxicity, which is associated with long-term neomycin therapy. Thus, we will use neomycin only for the first three days and then it will be replaced with ciprofloxacin. Ciprofloxacin will be an appropriate replacement as it is well known to be deeply disruptive to the gut microbiota [4]. For the antifungal, we have chosen to use fluconazole, which is used to treat a variety of fungal infections, because it is considered to be a safer option than other systemic antifungals such as amphotericin.

We know from our animal studies that the 16S rRNA gene copy number decreases after three days of therapy and returns to baseline five days after antibiotics are discontinued. Fourteen (14) days of therapy was chosen for this study so that there would be continued suppression of bacterial load to allow sufficient time for healing of the gastrointestinal tract. We chose standard adult dosing for the treatment of systemic or gastrointestinal infections (see Section 5.1 for dosages).

Participants in this study will not be required to stop any prior medications (including immunosuppressive medications), with the exception of probiotics and antibiotics. If potential participants are on prescribed antibiotics related to their IBD, they are required to stop those antibiotics in order to participant in this study. If potential participants are on antibiotics for a non-IBD related reason, they will be required to complete their current course before being enrolled in this study. Furthermore, participants already on steroids as part of their IBD regimen will be required to maintain their dosage and regimen during the 14 days (Days 1-14) of the study drug intervention. If dosage is increased during the 14 day study drug regimen, the participant will be considered to have started rescue therapy and study drugs will be stopped.

2 Study Objectives

2.1 Primary Objective

The primary objective of this study is to determine the efficacy of a novel gut microbiota-targeted therapeutic regimen, comprised of a bowel lavage and oral antibiotics with or without an antifungal, in the management of active CD that is refractory to conventional, immunosuppressive therapy.

2.2 Secondary Objectives

The secondary objectives will be to:

- Correlate effectiveness in reducing bacterial 16S and fungal 18S rRNA gene copy number, by the use of the regimen in the primary objective, with improvement in disease activity for patients with CD refractory to immunosuppression.

- Determine the relationship between the effectiveness of the proposed therapies with changes in gut microbiota composition

3 Investigational Plan

3.1 General Design

"Holiday" will be a randomized, placebo controlled, double blind Phase 2a trial, with an enrollment goal of 30 adult patients to yield an estimated 20 evaluable participants. Once 10 adult participants have completed the intervention phase, if there are an acceptable number and severity of adverse events, we will submit an amendment and joint protocol application to The Children's Hospital of Philadelphia (CHOP) for an ultimate enrollment goal of 30 adults and 30 children to yield 20 evaluable participants in each group. Recruitment will target CD patients who have demonstrated nonresponse or LOR to immunosuppressive therapy, and may be facing escalation of therapy or even surgery. Participants will be treated with bowel lavage and oral antibiotics with or without an antifungal over a period of 14 days. Clinical efficacy will be assessed at defined time points through scoring on the Harvey-Bradshaw Index (HBI) and the short Crohn's Disease Activity Index (sCDAI), both validated measures of CD disease activity, the fecal calprotectin (FCP), a clinical marker of intestinal inflammation, and the high-sensitivity C-reactive protein (hs-CRP), a marker of systemic inflammation. At the stool collection time points, the composition of the gut microbiota will also be determined using 16S gene qPCR, 18S gene qPCR, 16S gene sequencing, and internal transcribed spacer (ITS) gene sequencing to determine effects on bacterial and fungal load and microbiota composition. 16S gene qPCR determines bacterial load. 18S gene qPCR determines fungal load. 16S gene sequencing determines bacterial microbiota composition. ITS gene sequencing determines fungal microbiota composition.

3.1.1 Screening Phase

Potential participants will be identified through outpatient clinic schedules and iConnect, as well as physician referrals. For participants recruited through the outpatient clinic, written informed consent will be obtained at that outpatient visit, a REDCap Screening Questionnaire will be completed together, and consequently, an enrollment Clinical and Translational Research Center (CTRC) visit will be scheduled. Participants consented at an outpatient clinic visit will be given a stool collection kit, so that they may bring a stool sample to the CTRC visit. For participants identified as meeting inclusion criteria for the study and referred to the study team by their physician, participant contact will be made via telephone using an IRB approved pre-screening script to obtain verbal consent. Once verbal consent is obtained, potential participants will be emailed a REDCap Screening Questionnaire. Participants who seem to meet all inclusion criteria (see Section 4.1 and 4.2) will be scheduled for a CTRC visit and will be shipped a stool collection kit via mail. Written informed consent for participants identified through physician referrals will be obtained as the first step of the enrollment CTRC visit. This same sequence of events will take place for iConnect recruits. For all participants, the enrollment CTRC visit will include a physical

examination by a study investigator or CTRC nurse practitioner to determine eligibility based on clinical parameters. For eligible participants, blood and stool samples will also be obtained at the enrollment visit for baseline values. If necessary, (no documentation of a negative test within the previous 4 weeks), the stool sample will be tested to rule out *Clostridium difficile* infection. However, it will not be necessary to wait for the result of this test to begin the study regimen as vancomycin, one of the study medications, is standard treatment for *C. difficile* infection. **If a participant tests positive for *C. difficil*e infection at enrollment, they will be withdrawn from the study and their primary GI physician will be notified so that antibiotic therapy can be continued on a clinical basis.** The enrollment visit blood sample will also be assessed for serum creatinine to ensure no participants with impaired kidney function are actively enrolled. Female participants will have a urine pregnancy test. All participants will undergo electrocardiogram (EKG) to assess QTc interval at baseline. Participants will also be asked to complete 7 days of an online "Daily Survey" through REDCap to record their baseline symptoms before starting any study drug.

3.1.2 Intervention Phase

For participants who are fully eligible, medications will be shipped to the participant's home address. There will be no more than 17 days between the enrollment visit and the start of the intervention phase. The antimicrobial regimen will include 3 days of vancomycin and neomycin followed by 11 days of vancomycin and ciprofloxacin. Participants will also be randomized in a double-blinded fashion to fluconazole or placebo for 14 days. See Section 5.1 for details about dose, frequency and duration of all study drugs. Promethazine can be taken on an as needed basis on Days 1-3 for nausea secondary to neomycin.

On Day 2, participants will also undergo a bowel preparation with polyethylene glycol (PEG) 3350 (see Table 5.1). On this day, the diet will be limited to clear liquids. There will be no other dietary modifications during the course of the study.

On Days 1-14, participants will record their symptoms daily through a secure, web-based portal (REDCap). This information will be used to calculate the sCDAI score. In– person study visits will take place on days 5, 8, and 15. The indicated clinical and laboratory parameters will be assessed at the time of these visits. See table in Section 6 for details.

Serum creatinine levels of participants will also be intermittently monitored on Days 8, 15, and 29. At any time during the course of the intervention if a participant develops an elevated serum creatinine of greater than 1.3 in women and 1.5 in men or an increase of 0.25, study medications will be immediately stopped and participants will be brought in for an unscheduled visit to re-test their serum creatinine level. If their re-test results are within the normal range and/or less than 0.20 above their baseline, participants will resume study medications for the duration of the scheduled regimen, but if their levels are still elevated, they will be referred to their primary care physician (PCP) for clinical care. Events as described above will be considered an Adverse Event (AE) and there will be appropriate follow-up as detailed in Section 9 of this protocol to ensure that serum creatinine levels have normalized.

There is a +/- 1 day window within which the Day 15 visit must occur.

3.1.3 Optional Intervention Extension Phase (14 day repeat drug regimen)

Participants who achieve clinical response (reduction of the HBI by 3 or more points) or clinical remission (HBI < 5) to the study intervention at Day 15, but have relapsed (HBI ≥ 7 or sCDAI ≥ 220) between days 30 and 64 (2 months), will be offered the option to repeat the 14 day study regimen. Once the study coordinator is aware of a participant's disease relapse, the participant's symptoms will be observed for 2 weeks through the daily survey (but on a weekly basis) before starting their extension phase to ensure relapse. Participants who repeat the regimen will not require rescreening or a repeat enrollment visit as the repeated regimen will exclude fluconazole/ placebo, eliminating the risk of a prolonged QTc interval. The PEG lavage will also be excluded. An extra telephone visit (visit 6.1) will be added to the total number of the study visits. This visit will take place at the end of the extra 14 day regimen and so will vary

depending on the relapse date of each participant. All participants will have a 3 month visit (visit 7) and the last study visit for all participants will be at 6 months.

3.1.4 Follow Up Phase

The follow-up phase will include Day 16 until 6 months following the end of the intervention phase. The purpose of the follow-up phase is to determine the durability of the effect of the intervention over time. A telephone follow-up will occur on Day 22. An in-person study visit will take place on Day 29. The indicated clinical and laboratory parameters will be assessed at the time of these visits (See Table 1 in Section 6). There is a +/- 3 day window within which these two visits (Day 22 and 29) must occur. Finally, telephone follow-ups will occur at 3 months and 6 months following the intervention phase. There will be a 14-day (2 weeks) window in which the 3 month and 6 month follow-up telephone visits must occur for the data to be included in the analysis.

3.2 Study Endpoints

3.2.1 Primary Study Endpoints

The primary endpoint will be the change in disease activity, as measured by HBI score and FCP concentration, between the enrollment visit and Day 15*. All participants who withdraw for any reason prior to day 15 will be considered treatment failures.*

- Participants with an HBI < 5 at Day 15, and a FCP concentration ≤ 200 mcg/g at Day 15, will be defined as having achieved full clinical remission.
- Participants with an HBI < 5 at Day 15, but with elevated FCP (i.e. > 200mcg/g), will be considered responders.
- Participants with a decrease in HBI score of 3 or more points but HBI remains > 5 at Day 15, regardless of FCP concentration level, will be considered partial responders.

3.2.2 Secondary Study Endpoints

Secondary endpoints will include:

- The change in CRP (or hsCRP) between the enrollment visit and day 15
- Correlation of effectiveness in reducing bacterial 16S and fungal 18S rRNA copy number, by the use of oral antimicrobials combined with bowel lavage, with improvement of disease activity in patients with CD refractory to immunosuppression.
- The relationship between the effectiveness of the proposed therapies with changes in gut microbiota composition
- Safety and tolerability of the treatment regimen based on medication side effects and/or adverse events (AEs).

4 Study Population and Duration of Participation

4.1 Inclusion Criteria

- Participant is capable of giving informed consent
- Males or females 18-75 years of age
- Normal kidney function (defined by normal serum creatinine [male: <1.27mg/dL; female: <1.03mg/dL])

- Normal AST (<41U/L), ALT (<63U/L), and alkaline phosphatase (<126U/L)
- Active CD defined as HBI ≥ 7*

**Patients who have a self-report HBI of at least 6 (excluding the abdominal mass component of the index, which requires a physical examination by a clinician) will be considered eligible for scheduling of an enrollment visit and will only be actively enrolled with a definite HBI score of 7 or above, inclusive of the physical examination and abdominal mass component.*

- hs-CRP >10mg/L (or 1mg/dL) or fecal calprotectin (FCP) > 350mcg/g (within one month of enrollment)
- Have been treated with one of the following therapies** for at least 8 weeks with primary nonresponse or an initial response, followed by LOR (self-reported worsening of symptoms for ≥ 7 days): azathioprine, 6-mercaptopurine, methotrexate, adalimumab, certolizumab, golimumab, infliximab, natalizumab, vedolizumab, or ustekinumab
- If taking steroids, dose must be stable for at least 2 weeks prior to screening

***These medications must have been administered at standard, therapeutic dosages.*

4.2 Exclusion Criteria

- Unwillingness to provide informed consent
- Allergy or intolerance to the medications used in this study
- Pregnant or lactating females
- Known or suspected stricturing disease producing obstructive symptoms
- Active *Clostridium difficile* infection
- History of cirrhosis
- Baseline QTc interval on EKG > 430ms in males or > 450ms in females
- Current use of antibiotics
- Starting or increasing the dose of a IBD related medication within 4 weeks of screening including:
    - Azathioprine or 6-mercaptopurine (6MP)
    - Infliximab, adalimumab, certolizumab, or golimumab
    - Natalizumab or vedolizumab
    - Methotrexate
    - Any 5-ASA compound (e.g. Lialda, Asacol, etc)
    - Prednisone, budesonide or other steroids delivered orally or rectally
- Participants who, in the opinion of the investigator, may be non-compliant with study schedules or procedures

4.3 Participant Recruitment

Potential participants will be identified through outpatient clinic schedules and physician referrals from the Hospital of the University of Pennsylvania (HUP) or Penn Presbyterian Hospital (PPCM). The study will also be posted on iConnect and participants may be reminded to ask their primary GI provider or contact a study team member directly about this study through blasts in the MyPennMedicine portal. The team will procure weekly patient lists from the Penn Data Analytics Center (DAC) and target this population by sending out an IRB approved template email via MyPennMedicine. This has been approved by the OCR PennChart Research workgroup. Lastly, the study will also be posted on the following website: http://i3studypenn.weebly.com/associated-studies.html. An IRB approved flyer may also be posted on this website. Lastly, other web-based advertising avenues may be utilized in recruitment efforts including targeted advertising through local disease/health foundations such as the Crohn's and Colitis Foundation of America (CCFA). Recruitment will also be conducted using social media platforms such as Facebook and Twitter using IRB approved language and hashtags. If there are questions regarding initial eligibility, necessary information from the participant's medical record will be obtained by the treating physician and/or a member of the study team. Whenever possible, for participants recruited through the outpatient clinic, written consent will be obtained at that outpatient visit, a REDCap Screening Questionnaire will be completed, and participants will be scheduled for an enrollment CTRC visit. For participants recruited through physician referrals, patients will be contacted and screened via telephone using an IRB approved pre-screening script to obtain verbal consent. Once verbal consent is obtained, potential participants will be emailed a REDCap Screening Questionnaire. Participants who seem to meet all inclusion criteria (see Section 4.1 and 4.2) will be scheduled for a CTRC enrollment visit. Crohn's disease history will be obtained. A stool collection kit will be given to or mailed to all participants so that a sample can be brought to the enrollment CTRC visit. No samples will be received or analyzed before obtaining written consent.

All participants must provide written consent before undergoing any other study procedures. The study procedures will be explained to each participant at the time of pre-screening and written consent. If the participant agrees to further participate, the written informed consent form will be signed in-person at either the outpatient clinic visit or the enrollment CTRC visit.

4.4 Duration of Study Participation

The study duration per participant will be approximately 6-8 months, with up to 30 days for screening (from telephone verbal consent to the enrollment visit), 2 weeks for the intervention phase, and up to 6 months for the follow-up phase.

Participants who meet criteria and opt to undergo the extension phase of the antimicrobial regimen will prolong their participation in the study by approximately 2 weeks.

4.5 Total Number of Participants and Sites

The study will be conducted at the clinical centers at the University of Pennsylvania (HUP and PPMC). Recruitment will stop when approximately 20 participants are fully eligible. It is expected that approximately 30 participants will be enrolled (screened) to produce 20 evaluable participants.

4.6 Vulnerable Populations:

Children, pregnant women, fetuses, neonates, or prisoners are not included in this research study.

5 Study Intervention

5.1 Intervention Description and Regimen

Drug	Dosage Form	Dosage	Regimen
Vancomycin	500 mg oral suspension	500 mg (1 syringe) every 6 hours	Days 1-14

Neomycin	500 mg tablet	1 g (2 tablets) PO every 8 hours	Day 1-3
Ciprofloxacin	750 mg capsule	750 mg (1 capsule) PO every 12 hours	Days 4-14
Encapsulated fluconazole or placebo capsule	100 mg tablet encapsulated	400 mg (4 capsules) PO daily	Days 1-14
Miralax (PEG 3350)	Powder	238 g dissolved in 64 oz liquid with electrolytes	Day 2
Promethazine	Tablet	12.5mg PO as needed every 4 hours	Days 1-3

5.2 Randomization

Stratified randomization to fluconazole versus placebo will be performed using a 1:1 ratio of treatment arms. The randomization order will be created by University of Pennsylvania's Investigational Drug Service (IDS)

5.3 Receipt

After confirmation of eligibility, participants will be sent the antimicrobial and lavage regimen listed above directly from IDS to their home mailing address. All study medications will be shipped in one parcel via UPS. Participants will be provided with a checklist of all study medications and will be instructed to report any discrepancies to a member of the study team.

5.4 Storage

Study medications will be prepared, stored, and dispensed by IDS under controlled conditions.

5.5 Preparation and Packaging

To promote participant compliance and minimize potential confusion, study medications will be packaged into 2 blister packs: one for Days 1-7 and the other Days 8-14. The rows of the blister packs will be labeled as "Day 1" "Day 2" etc., through "Day 14", with each dose separated into blisters by meal times. The vancomycin suspensions will be packaged in separate Ziploc bags per day. Each bag will contain four syringes containing 500 mg/20 mL suspension vancomycin. The Day 2 bag will additionally contain a bottle with 238 g of PEG 3350. At the enrollment visit, participants will be provided with a 64 ounce water pitcher plus either 2 32 ounce bottles of Gatorade or packets of Crystal Lite. This will depend on participant preference. However, for participants who have diabetes, Crystal Lite will be required.

With the exception of vancomycin and fluconazole, all study medications will be distributed in their original formulation as per the manufacturer. For vancomycin, IDS will compound 1,120 mL of oral suspension (500 mg/20 mL concentration) drawn up into 56 syringes per participant (see Appendix B). For fluconazole, IDS will encapsulate the original formulation tablets so that they will look identical to placebo capsules.

5.6 Administration and Accountability

Participants will be provided with all study medications by tracked courier (UPS) to their home address following the enrollment visit and consequent confirmation of their eligibility. Participants will take all medications orally. At the final in-person study visit, all unused study medications and empty containers will be collected from each participant.

5.7 Participant Compliance Monitoring

At all encounters following the enrollment visit, compliance will be monitored through study staff observation of empty blister packs at each in person visit (see Section 5.7). At each in-person visit, participants will be asked to bring in their used and unused blister packs, syringes, and PEG bottle for review by study staff. Compliance will be reviewed with the participants at each in-person visit.

5.7.1 Return or Destruction of Investigational Product

Records of study drug receipt and disposition will be maintained by the IDS. Investigational drug orders and dispensing records will be examined quarterly by a study coordinator. The study medication will be prescribed by Study Investigators and may not be used for any purpose other than that described in this protocol. At study completion, all drug supplies including partially used and empty containers must be returned to Investigator or a study coordinator.

5.8 *Unblinding procedure*

At the end of the enrollment visit, eligible participants will be randomly assigned to one of the two treatment groups (fluconazole or placebo) according to the randomization schedule generated by Penn IDS. Neither the participant, the study team, nor the clinical site personnel will know the treatment group to which any participant is randomized.

If there is a serious adverse event, which is thought by the study team to be possibly or probably related to the coded medication, the principal investigator, when necessary for the safety of the participant, will unblind treatment group assignment. The following procedures will be taken when the sponsor or principal investigator deems that unblinding is necessary: The IDS will be notified by the study team that unblinding is necessary. IDS will unblind the treatment group assignment and provide it to the principal investigator and the study coordinator.

Unblinding of treatment assignment is anticipated to be an uncommon occurrence and is highly discouraged. Unblinding should only be performed if deemed necessary for the safety of the participant.

6 Study Procedures

Table 1: Schedule of Study Procedures

	Screening/ Recruitment	**Enrollment/ Day 0**	**Day 5**	**Day 8**	**Day 15**	**Day 22**	**Day 29**	**Day 36-64 (Optional visit)**	**3 mos**	**6 mos**
Study Visit	Visit 0	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6	Visit 6.1	Visit 7	Visit 8
Phase of Study			Intervention Phase						Follow-up Phase	
Telephone visit	X					X		X	X	X
Web-based screening survey	X									
Daily Survey			X	X	X	X	X	X (weekly)	X (weekly)	X (weekly)
Medical record review	X	X							X	X
Physical exam including vital signs		X	X	X	X		X			
Urine pregnancy test for female participants		X								
Stool collection for microbiome analysis		X	X	X	X	X	X			
Stool *C. difficile* toxin	X (within 1 month)	X (if not done within 1 month)								
Rectal swabs for mucosal analysis		X		X	X		X			
FCP	X	X		X	X	X	X			
CBC (no diff)		X	Blood draw to store for future	X	X		X			
CMP		X		X	X		X			
ESR		X		X	X		X			

hsCRP		X	use	X	X		X			
Infliximab or adalimumab level**		X								
HBI	X (within 1 month)	X	X	X	X		X			
EKG		X		X						
	**For participants on infliximab or adalimumab									

6.1 Screening Phase (Visit 0)

Potential participants will be identified through outpatient clinic schedules as well as physician referrals. For participants recruited through the outpatient clinic, written consent will be obtained at that outpatient visit and participants will be scheduled for an enrollment CTRC visit. Screening for these participants will proceed as follows:

- Written informed consent at outpatient clinic visit
- Email or complete with coordinator screening questionnaire through REDCap. If seems eligible, schedule enrollment CTRC visit
- Distribute stool kit in-person at outpatient clinic visit or via courier service (UPS)

Or for participants identified through physician referrals or iConnect, potential participants will be contacted and screened after obtaining verbal consent via telephone using a pre-screening script, emailed a screening questionnaire and scheduled for an enrollment CTRC visit. Screening for these participants will proceed as follows:

- Receive physician referral or iConnect alert
- Contact potential participant via telephone, obtain verbal consent, review study procedures and eligibility criteria
- For applicable and interested participants, email screening questionnaire through REDCap. If seems eligible, schedule an enrollment CTRC visit
- Distribute stool kit via courier service (UPS)

6.2 Enrollment Phase (Visit 1)

Eligible participants will be seen by a study team investigator and study coordinator at the CTRC for the enrollment visit. The written informed consent form may be signed at this visit if not already completed. Enrollment will proceed as follows:

- Written informed consent (if applicable)
- Review and documentation of eligibility criteria
- Obtain collected stool sample (kit provided during screening phase) for analysis of *C. difficile* toxin, fecal calprotectin, and microbiome
- Vital signs
- Anthropometric measurements
- Physical exam, including CD disease symptom assessment for HBI
- Rectal swabs (2) for mucosally-associated microbiome analysis
- Blood sample (CBC, CMP, hsCRP, ESR, biologic levels if applicable with extra stored for future use)
- Urine pregnancy test for female participants
- EKG
- Participant counseled to continue all current medications *except* probiotics
- Medication education
- Distribution of Gatorade or Crystal Lite (and a pitcher)

On the same day as enrollment, one (1) stool collection kit will be given to each participant so that a sample can be brought to us on Day 8.

6.3 Intervention Phase (Days 1-15 including Visits 2-4): In-person Study Visits

Day 1 is defined as the day that a participant starts the study drug regimen. Regardless of the day that participants receive the study drugs in the mail from IDS, they will be instructed call the coordinator, who will instruct them to start taking study drugs on the upcoming Monday, Thursday, or Friday, in order to enhance compliance and ensure that in-person study visits do not fall on weekends. This was also allow coordinators to track medication packages.

6.3.1 Daily Surveys

After verbal and/or written consent is obtained from participants, they will be asked to complete 7 days of online daily surveys via REDCap before Day 1 to obtain baseline data. From Day 1 through Day 29 of the study (intervention phase and first 15 days of follow-up phase), participants will complete the same daily survey through REDCap so that the study team can monitor disease activity by the sCDAI. Participants will be prompted to identify any concerns including medication side effects and/or worsening of symptoms. From Day 30 through 6 months, the survey including disease activity questions only will be completed once weekly. On in-person study visit days, the daily survey may be completed with the study coordinator during the visit.

Survey responses will be monitored by study staff through REDCap on a regular basis. If more than two (2) daily surveys are not completed in a week, the coordinator will contact the participant via phone and/or email to ensure compliance. If a weekly survey is not completed, participants will also be contacted by the study coordinator.

If a participant is enrolled in the study that does not have daily access to the internet and/or email, a daily survey paper diary will be provided to them at the start of their study participation. This will include all the REDCap daily (and weekly) surveys. In order to ensure compliance for this group, participants will be required to ship the completed paper surveys to the study team on a weekly basis. UPS or FedEx shipping labels will be provided in advance to enable this practice.

6.3.2 Study Visit 2 (Day 5)

Study Visit 2 will take place on Day 5. Participants will be seen at the Penn CTRC by a study coordinator and/or CTRC nurse. The following will be required:

- Vital signs
- Anthropometric measurements
- Physical exam, including CD disease symptom history for HBI
- Blood sample for future use (metabolomics and neomycin levels)
- Collect stool sample (kit provided at previous visit)
- Adverse event assessment (see Section 9)
- Study drug compliance assessment
- Update current medications log
- Daily Survey (optional)

6.3.3 Study Visit 3 (Day 8)

Study Visit 3 will take place on Day 8. Participants will be seen at the Penn CTRC by a study coordinator and/or CTRC nurse. The following will be required:

- Vital signs
- Anthropometric measurements
- Physical exam, including CD disease symptom assessment for HBI
- EKG
- Rectal swab for mucosally-associated microbiome analysis
- Blood sample (CBC, CMP, hsCRP, ESR with extra stored for future use)
- Collect stool sample (kit provided at previous visit)
- Adverse event assessment (see Section 9)

- Study drug compliance assessment
- Update current medications log
- Daily Survey (optional)

On Day 8, one (1) stool collection kit will be given to each participant so that a sample can be brought to us on Day 15.

6.3.4 Study Visit 4 (Day 15)

Study Visit 4 will take place on Day 15. Participants will be seen at the Penn CTRC by a study coordinator and/or CTRC nurse. The following will be required:

- Vital signs
- Anthropometric measurements
- Physical exam, including CD disease symptom assessment for HBI
- Rectal swab for mucosally-associated microbiome analysis
- Blood sample (CBC, CMP, hsCRP, ESR with extra stored for future use)
- Collect stool sample (kit provided at previous visit)
- Adverse event assessment (see Section 9)
- Study drug compliance assessment and collect any unused study drug
- Update current medications log
- Daily Survey (optional)

On Day 15, two (2) stool collection kits will be given to each participant so that 1) a sample can be collected and mailed to us by Day 22 and 2) a sample can be brought to us on Day 29 (Study Visit 6).

6.4 Follow-Up Phase (Days 16- 6 months including Visits 5-8): Telephone and In-person Study Visits

The follow-up phase will include Day 16 through 6 months following the end of the intervention phase. Telephone follow-up will occur on Day 22 (see Section 6.4.1). An in-person study visit will take place on Day 29. Day 30 onwards, until 6 months, participants will continue to record symptoms in REDCap, *once weekly*. Telephone follow-up will occur at 3 months and 6 months following the beginning of the intervention.

6.4.1 Telephone Follow-Up: Visit 5 (Day 22)

A telephone follow-up will be conducted on Day 22 by the study coordinator. During the telephone session, questions will be asked to assess the general state of their Crohn's Disease and their current medication regimen. The coordinator will also look at their electronic medical record to record any recent surgeries, hospitalizations, and C diff. test results since their last study visit. Participants will be reminded to ship a stool sample collected within 24 hours of this call

6.4.2 Study Visit 6 (Day 29)

Visit 6 will take place on Day 29. Participants will be seen at the Penn CTRC by a study coordinator and investigator or CTRC nurse. The following will be required:

- Vital signs
- Anthropometric measurements
- Physical exam, including CD disease symptom assessment for HBI
- Rectal swab for mucosally-associated microbiome analysis
- Blood sample (CBC, CMP, hsCRP, ESR with extra stored for future use)
- Collect stool sample (kit provided at previous in-person visit)
- Adverse event assessment (see Section 9)
- Study drug compliance assessment
- Update current medications log

- Daily Survey (optional)

6.4.3 Telephone Follow-Ups: 3 Months & 6 Months

Telephone follow-up will also occur at 3 months and 6 months after the intervention phase. For these follow-up sessions, we will allow a two-week window. During the telephone session, questions will be asked to assess the general state of their Crohn's Disease and their current medication regimen. The coordinator will also look at their electronic medical record to record any recent surgeries, hospitalizations, and C diff. test results since their last study visit.

6.5 Rescue Therapy

If a participant's condition worsens during the course of the study, the PI in conjunction with the participant's primary provider will make a decision, based on clinical expertise, to withdraw the participant from the study. If the participant receives a rescue medication after the study intervention regimen is completed (Day 15 or later) the participant has the option to continue to participate in the study, at his or her discretion. For participants who need to receive rescue medication(s) during Days 1-14 of the study from their primary GI physician, study drug will be stopped and they will be considered to have not responded to therapy. With their permission, these participants will be followed to the end of the 6 month time point of the study schedule.

6.6 Participant Withdrawal

Participants may withdraw from the study at any time without prejudice to their clinical care. They may also be discontinued from the study at the discretion of the Investigator for lack of adherence to study treatment or visit schedules, AEs, lack of response, reasons of safety, administrative reasons and/or need for rescue medication during the enrollment or intervention phase. Participants who require rescue medication or surgery after completion of the intervention regimen (Day 15 or later) will continue to be followed by the study with their permission. It will be documented whether or not each participant completes the clinical study. If the Investigator becomes aware of any serious, related adverse events after the participant completes or withdraws from the study, they will be recorded in the adverse event log of the study.

6.7 Early Termination Visits

If a participant would like to withdraw their participation, research study staff will document when and why the participant withdrew from the study. In this case, no further action will be needed. Participants will be made aware in the consent process that data collected prior to their withdrawal may still be used for study analysis. Participants who withdraw consent to participate in the study or who are withdrawn by the principal investigator during the intervention phase will be seen for one final in-person visit to collect the investigational product.

7 Study Evaluations and Measurements

7.1 Medical Record Review

The following is a list of elements that may be abstracted from the participant's medical chart (either paper or electronic):

- Date of birth
- MRN
- Mailing address
- Email address
- Telephone number
- Sex
- Race
- Most recent height

- Most recent weight
- Crohn's disease history (including date of diagnosis, and disease location)
- Prior use of medications
- Current medications
- Medication allergies
- Surgical history (including all surgeries regardless of relation to IBD)
- Co-morbid medical conditions
- History of stricturing or fistulizing disease (from review of records for history of bowel obstruction and imaging studies)
- Patient report of bowel frequency
- Patient report of abdominal pain
- Patient report of general well-being
- Most recent physical examination
- Laboratory tests (including ESR, CRP, albumin, hematocrit, hemoglobin, fecal calprotectin, stool C. difficile toxin, drug levels for biologics)

7.2 Physical Examination

A physical examination will be performed in order to calculate the HBI at all in-person study visits. A study investigator or CTRC nurse practitioner will document whether there is an abdominal mass (0=none, 1=dubious, 2=definite, 3=definite and tender). A study investigator or CTRC nurse practitioner will also document any extraintestinal manifestations of disease, including arthralgia, uveitis, erythema nodosum, aphthous ulcers, *Pyoderma gangrenosum*, anal fissure, new fistula, and/or abscess. A brief overall physical exam may also be completed with documentation being completed in EPIC by creating an "out of office" encounter. A copy of this documentation should be printed out from EPIC and added to the participant's study file.

7.3 Vital Signs

Oral temperature will be obtained using a digital thermometer. Heart rate and blood pressure will be obtained using an automated device while the patient is seated. Height and weight will also be obtained. Height will only be measured once at the enrollment visit. All anthropometric measurements will be conducted with the participants wearing light clothing without shoes. Weight (0.1 kg) will be measured on a digital electronic scale (Seca, Munich, Germany), and stature (0.1 cm) on a stadiometer (Holtain, Crymych, UK). Clinical anthropometric measurements taken may be used for the study as well.

7.4 EKG

A 12-lead EKG will be performed by trained CTRC staff on Day 0 (the enrollment visit) and on Day 8 (Visit 3). EKG is indicated because both ciprofloxacin and fluconazole carry some risk of QTc interval prolongation. The QTc interval will be assessed by a study investigator using manual determination with a caliper. A prolonged QTc interval is defined as >430msec in males and >450msec in females. Participants with a prolonged QTc interval at baseline will be excluded. Participants with a prolonged QTc interval on Day 8 will discontinue the fluconazole/ placebo aspect of the study medication regimen. The study coordinator will remove the fluconazole/ placebo from the blister pack to avoid accidental doses.

7.5 Laboratory Blood Evaluations

Blood will be collected by trained CTRC staff and processed either by the Hospital of the University of Pennsylvania clinical laboratory (Pepper Lab) or by ARUP laboratories. The following testing will be performed:

- ESR
- hsCRP
- CMP
- CBC without differential
- *Drug levels for participants on biologics stored for future testing

- **Extra blood for storage for future testing

* At the enrollment visit, blood will be obtained for drug level and antibody testing for patients who are being treated with infliximab or adalimumab (ARUP laboratories). Serum will be stored frozen in ARUP transport tubes. Serum will be stored for future use so that it will be possible to correlate response to this regimen with biologic drug levels and presence or absence of biologic medication antibodies.

**An extra 8mL of blood will be drawn and will be stored for future metabolomics studies at the enrollment visit, day 5, day 8, day 15, and day 29.

7.6 Pregnancy Testing

A urine pregnancy test will be performed at the enrollment visit in the CTRC for all female participants.

7.7 Stool Collection, Shipping, and Analysis

Each participant will be provided with five (5) stool collection kits to take home during the course of the study. Each kit will contain a collection hat, ice packs, shipping bag, a box to hold the collection container, a specimen bag, and a prepaid shipping label (for the one specimen that will be shipped). Each kit will also include a small spoon top vial with pre-measured ethanol to preserve the freshest stool sample. Samples being shipped with an ethanol aliquot will be appropriately packaged with an "Excepted Quantities" label. This aliquoting will be completed by the participants before shipping the sample. Stool collection and aliquoting instructions will be provided and verbally explained to all participants in advance. The participants will also be asked to complete a form identifying their sample from the standard Bristol Stool Chart.

Participants will be instructed to collect a bowel movement at 5 time points – enrollment, Day 8, Day 15, Day 22, and Day 29. Stool samples must be collected no more than 24 hours before an in-person visit delivery time or being picked up by UPS for shipping. The Day 22 sample will be shipped to the study team. All other samples will be delivered to the study team on the day of the in-person study visits. For Day 22, participants will be counseled that stool samples should not be collected or shipped Friday-Sunday. Alternatively, the participant may also choose to deliver the Day 22 sample to a study team member in-person.

All specimens remaining after the study is complete will be retained for possible future use unless the participant requests that the specimen be destroyed (opt-out) or it is deemed by the investigators that the specimens are no longer needed.

An aliquot of stool from the enrollment sample will be sent to Penn microbiology laboratory for C. difficile testing if necessary (not already obtained clinically as previously described). An aliquot of stool from each of the 5 stool samples will be sent to a study lab for FCP testing. The remaining stool will be aliquoted and stored in the laboratory of Dr. Gary Wu (sub-investigator). The tube containing ETOH will be stored at -80°C. The excess stool will be aliquoted as follows and stored at -80°C: 4 spoon-top tubes (Sarstedt) – 3 dry and 1 containing RNALater®, 1 coring tube (Globe Scientific) to obtain frozen stool cores for use with CryoXtract 350. All analyses will occur in Dr. Wu's laboratory or at the PennCHOP Microbiome Center.

7.8 Rectal Swabs

At the enrollment visit, Study Visit 3, Study Visit 4, and Study Visit 6, participants will undergo a rectal swab for assessment of the mucosally-associated gut microbiota. Based on results from the Stool/Swab Pilot study (CHOP IRB #11-008506), differences were identified in the composition of the gut microbiota in rectal swabs as compared to traditional stool samples. Thus, we will study microbial differences between collected rectal swabs and stool samples [5].

Rectal swabs will be obtained during the physical examination by the CTRC nurse practitioner or study investigator. Two (2) sterile swabs will be inserted 3 cm into the rectum, turned 360 degrees, removed,

placed into a sterile tube, and frozen at -80 degrees celcius until analysis. One (1) swab will be waved in the air and collected as a control.

For the first 5 subjects, one of the swabs at each time point will be delivered immediately to Dr. Wu's laboratory for anaerobic culture. This is in order to validate microbiome PCR results with anaerobic culture.

All frozen swabs will be analyzed by the PennCHOP Microbiome Center.

7.9 Efficacy Evaluations

7.9.1 HBI and short CDAI scoring

The Harvey-Bradshaw Index (HBI) is a validated, non-invasive scale that is widely used in clinical trials to assess Crohn's disease activity among adults [31, 32]. Devised as a "simple" but accurate alternative to the full Crohn's Disease Activity Index (CDAI), which requires patients to recall and average daily symptoms over the course of a week, the HBI scale incorporates self-reported symptoms and physical examination findings for an overall score of 0 to >16 [31]. In accordance with clinical trial standards, the current study will define active disease as an HBI score ≥ 7. To be defined as having reached full clinical remission, patients must achieve an HBI score < 5, and a FCP concentration ≤ 200 mcg/g. Participants with HBI < 5 but with elevated FCP (i.e. > 200 mcg/g) will be considered partial responders. Participants who achieve clinical response with a reduction in HBI score of three or more, but without remission (HBI < 5), will also be considered partial responders. The short CDAI (sCDAI), a modified, validated score of disease activity that includes only patient self-report elements [33], will be administered through the daily REDCap surveys on Day 1-29 and then weekly thereafter (see Section 6.3.1).

7.9.2 Fecal calprotectin

Evidence suggests that fecal calprotectin (FCP) represents an excellent surrogate marker of intestinal inflammation and disease activity. Calprotectin is 36 kDa calcium- and zinc-binding protein that represents 60% of cytosolic proteins in granulocytes [34]. Furthermore, calprotectin is highly stable in feces when stored at room temperature for up to 1 week [6]. Clinically, the concentration of calprotectin in feces (FCP) is used as a non-invasive measure of neutrophilic infiltrate in the bowel mucosa, and thus intestinal inflammation. The correlation of decreased FCP concentration to mucosal healing has been demonstrated by endoscopy in ulcerative colitis and Crohn's disease [7]. This has been confirmed in both adult and pediatric populations with Crohn's disease [8, 9]. A recent meta-analysis identified a cut point of 250 mcg/g as optimal to distinguish the presence or absence of endoscopically detectable mucosal inflammation [40]. However, reduction in FCP is also used to assess improvement. For example, following treatment with anti-TNF therapy, FCP has been demonstrated to dramatically decline [41].
In this study, FCP concentration will be assessed using ELISA methods (study labs).

7.9.3 High-sensitivity C-reactive protein (hs-CRP)

C-reactive protein is produced mainly in hepatocytes in response to acute phase stimuli such as inflammation. Its production is driven by circulating cytokines. C-reactive protein is commonly used to screen the activity of chronic inflammatory diseases including IBD. In general, patients with CD have a high CRP when the disease is active and a normal CRP when the disease is quiescent. High-sensitivity CRP (hs-CRP) assays measure CRP levels that were previously thought to be under detection limits. Hs-CRP levels are thought to correlate with disease activity in IBD. Hs-CRP will be tested through Pepper Labs at the University of Pennsylvania.

7.9.4 Microbial DNA sequencing

DNA will be prepared in the PennCHOP Microbiome Center. Samples will be sent to the center de-identified and coded with a study number. We will perform 16S rRNA and ITS gene sequencing to evaluate the bacterial and fungal microbiota, respectively, in stool and rectal swab samples. Isolated DNA will be quantified using the Picogreen system and 50 ng of DNA will be amplified. Pyrosequencing will be

carried out using barcoded primers as previously described [10]. For pyrosequencing of bacteria, primers annealing to the V1V2 region of the 16S bacterial gene will be used. The development of the ITS1 fungal primers is described in [10]. For pyrosequencing, we will use the Roche/454 Genome Sequencer Junior. Sequence data will be processed using QIIME [11]. If warranted by our preliminary data, we may analyze samples further using a metagenomic approach, in which DNA samples are nebulized, ligated to linkers, and subjected to pyrosequencing (IlluminaHiSeq). This allows enumeration of the types of genes present in a sample. To determine bacterial and fungal load, respectively, we will determine 16S and 18S gene copy number from the stool and rectal swab samples. The qPCR methods including the details of the primers as well as PCR cycling conditions have been previously described [12].

7.10 Safety Evaluations

Participant safety will be monitored by monitoring adverse events, medication side effects, vital signs, physical examinations, and laboratory data. The Principal Investigator and study team will specifically monitor participants for fever, nausea/vomiting, increased abdominal pain, and increased diarrhea from baseline (see Section 9). Adverse events will be compared to all known side effects of the antimicrobials prescribed through the current study (see Appendix A). All adverse events will be tracked and assessed by the PI. Adverse events will be reported according to Penn Research Policy and Procedure – please see Section 9.5 for additional safety monitoring details for this study.

8 Statistical Plan

8.1 Primary Endpoint

The primary endpoint will be the change in disease activity, as measured by HBI and FCP concentration, between the enrollment visit and Day 15*.All participants who withdraw for any reason prior to day 15 will be considered treatment failures.*

- Participants with an HBI < 5 at Day 15, and a FCP concentration ≤ 200 mcg/g at Day 15, will be defined as having achieved full clinical remission.
- Participants with an HBI < 5 at Day 15, but with elevated FCP (i.e. > 200mcg/g), will be considered responders.
- Participants with a decrease in HBI score of 3 or more points but HBI remains > 5 at Day 15, regardless of FCP concentration level, will be considered partial responders.

8.2 Secondary Endpoints

Secondary endpoints will include the following:

- The change in CRP (or hsCRP) between the enrollment visit and Day 15
- Correlation of effectiveness in reducing bacterial 16S and fungal 18S rRNA copy number, by the use of oral antimicrobials combined with bowel lavage, with improvement of disease activity in patients with CD refractory to immunosupression.
- The relationship between the effectiveness of the proposed regimen with changes to the composition of the gut microbiota
- Safety and tolerability of the treatment regimen based on medication side effects and/or adverse events (AEs).

8.3 Statistical Methods

The two treatment groups will be compared using standard descriptive statistics. Categorical variables will be compared using Fisher's exact test and continuous variables will be compared using the unpaired t-test or Wilcoxon rank sum test if the data are not normally distributed.

Our estimates of efficacy will use four outcome measures: clinical response, clinical remission, reduction in FCP concentration, and reduction in hsCRP. For clinical response (reduction of score on HBI by 3 or more points) we will report the proportion and binomial 95% confidence intervals. Similar methods will be used for remission (HBI < 5). Although 250 mcg/g has been recently recommended, there is no standard definition of a clinically meaningful reduction in FCP and many definitions have been used. Therefore, we will use a paired t-test (after applying a log transformation if necessary) to establish whether the FCP concentration is lower following therapy than at baseline and we will also report the proportion of patients with reduction in FCP to less than 250 mcg/g among the subset with baseline FCP > 350 mcg/g. Similarly, there is no standard definition of a clinically meaningful reduction in hsCRP. Therefore, we will use a paired t-test (after applying a log transformation if necessary) to establish whether the hsCRP is lower following therapy than at baseline.

As a Phase 2a study to determine the effectiveness of the two treatment arms to induce a clinical response or remission, with 20 participants, it is possible to generate the required data [43].

To detect reduction in bacterial and fungal load by 16S and 18S gene copy number we will use a paired t-test after applying a log transformation. Based on our preliminary data, we assumed that the standard deviation of change will be approximately 0.4 log and that a minimum of a 2 log drop in copy number would be clinically significant. To have 90% power, this requires only 3 participants. Even if the standard deviation is greater, say 1 or 2 log, the required sample size is 5 or 13, respectively. We will measure the Pearson's correlation to determine the relationship between copy numbers and the outcome measures listed above (HBI, FCP, and hsCRP). If all patients do not have >2 log drop in 16S and 18S copy number, we will compare clinical remission and response among those with and without >2 log drop using Fisher's exact test.

Our main statistical and computational tool for comparing the bacterial gut microbiota among different groups (e.g. between samples from different time points) is the phylogenetic-based method as implemented in the program UniFrac [46, 47], which measures the similarity among the community based on phylogenetic distances determined by the 16S rRNA gene sequences of different bacteria. Based on these distances, we can cluster the microbiomes using the Principal Coordinate Analysis (PCoA) along axes of maximal variance. The significant principal components can then be compared between two groups using the two sample t-tests. Permutations can be used to obtain the p-values. Alternatively, we can compare several principal components simultaneously by performing nonparametric permutation test for association between the two groups and the microbiome compositions. Specifically, we can randomize the labels of the groups and compare all distances between points that both come from the same group to all distances between points from different groups using t-tests. In such permutation test, we can obtain a nonparametric distribution of the t statistic that takes into account the correlations introduced by the pair-wise distance matrix structures. For the fungal community distances, Jaccard and abundance-weighted Jaccard indices will be calculated.

8.3.1 Safety Analysis

All participants entered into the study at the enrollment visit will be included in the safety analysis. The frequencies of AEs by type, body system, severity and relationship to study drug will be summarized. SAEs (if any) will be described in detail.

AE incidence will be summarized along with the corresponding exact binomial 95% two-sided confidence intervals.

We expect that adverse events will occur because the participants included in this study will have active, refractory disease. Thus, hospitalizations or even life-threatening events may occur. However, we anticipate very few, if any, adverse events that are directly related to the proposed intervention.

9 Safety and Adverse Events

9.1 Definitions

9.1.1 Adverse Event

An adverse event (AE) is any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related.

All AEs (including serious AEs) will be noted in the study records and on the case report form with a full description including the nature, date and time of onset, determination of non-serious versus serious, intensity (mild, moderate, severe), duration, causality, and outcome of the event.

9.1.2 Serious Adverse Event

A serious adverse event (SAE) is an adverse event that, in the view of either the investigator or sponsor, results in any of the following outcomes:

- death,
- a life-threatening adverse event,
- inpatient hospitalization or prolongation of existing hospitalization,
- a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions, or a congenital anomaly/birth defect

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

9.2 Recording of Adverse Events

At each contact with the participant the Principal Investigator and other study personnel will seek information on adverse events by specific questioning and, as appropriate, by examination. Information on all adverse events will be recorded immediately in the adverse event log (AE- CRF). All clearly related signs, symptoms, and abnormal diagnostic procedures results should recorded in the log, though should be grouped under one diagnosis.

All adverse events occurring during the period will be recorded. The clinical course of each event will be followed until resolution, stabilization, or until it has been determined that the study intervention or participation is not the cause. Serious adverse events that are still ongoing at the end of the period will 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 intervention or study participation will be recorded and reported immediately.

9.3 Relationship of AE to Study

The relationship of each AE or SAE to the study intervention will be characterized by the PI, with input from the study team as necessary, using one of the following terms in accordance with Penn IRB Policies: definitely related, probably related, possibly related, unlikely or unrelated.

9.4 Reporting of Adverse Events and Unanticipated Problems

Adverse events, serious adverse events, unanticipated problems, and adverse reactions will be reported to the Penn IRB through the HS-ERA system. Events that qualify for expedited reporting per Penn IRB Reporting Requirements will be reported within 3 or 10 business days, depending on the categories in the table below. All other events that do not fit expedited reporting requirements will be reported at the time of Continuing Review submission.

9.4.1 Study SAE Notification to Investigator

An SAE must be reported to the study investigators by telephone within 24 hours of the event. An SAE Form must be completed by the investigator. The investigator will keep a copy of this form on file at the study site. Report SAEs by phone or pager to:

James D. Lewis, MD, MSCE
Phone – (215) 573-5137
Cell – (856) 906-3173

In the event that Dr. Lewis cannot be reached report SAEs to
Lindsey Albenberg, DO
Phone - (215) 590-1680

Or

Unmesha Roy Paladhi
Phone – (215) 746-7138
Cell – (267) 788-8417

At the time of the initial report, the following information should be provided:

- Study Name
- Participant number
- A description of the event
- Date of onset
- Current status
- The reason why the event is classified as serious

Within the following 48 hours, the investigator must provide further information on the SAE in the form of a written narrative. This should include a copy of the completed SAE Form and any other diagnostic information that will assist the understanding of the event. Significant new information on ongoing SAEs should be provided promptly to the study investigator.

9.4.2 Penn IRB Safety and Expedited Reporting Requirements

Unanticipated Problem/Adverse Event Classification			**Penn IRB Reporting Requirements All Studies**
Research Related	Unexpected	Fatal/Life Threatening	
X	X	X	**≤ 3 working days**
X	X		**≤ 10 working days**
Other Events: • New information showing increased risk to participants • Unapproved protocol deviation to assure protection of human participants • Protocol deviation that places participants at risk or has the potential to occur again • Any serious or continuing non-compliance • Breach of confidentiality • Incarceration of participant • Withdrawal from marketing for safety concerns of the research drug,			**≤ 10 working days**

device, or biologic	

9.4.3 Follow-up report

If an SAE has not resolved at the time of the initial report and new information arises that changes the investigator's assessment of the event, a follow-up report including all relevant new or reassessed information (e.g., concomitant medication, medical history) should be submitted to the Penn IRB. The investigator is responsible for ensuring that all SAE are followed until either resolved or stable.

9.4.4 Investigator Reporting: Notifying the Penn IRB

The Investigator will promptly notify the Penn IRB of all on-site, unanticipated SAEs that are related to the research activity. Other unanticipated problems related to the research involving risk to participants or others will also be reported promptly. Written reports will be filed using the HS-ERA system and in accordance with the Penn IRB Policies (see Table 9.4.1). External SAEs that are both unexpected and related to the study intervention will be reported promptly after the Investigator receives notification.

9.5 Medical Monitoring

The current study has received an Investigational New Drug (IND) Exemption from the Penn IND/IDE Support Unit within the Office of Clinical Research. As part of an established safety and monitoring plan, the Primary Investigator will monitor adverse events and unanticipated problems during the study to monitor ongoing participant safety. A **Safety Officer, David S. Goldberg, MD, MSCE (646-242-6349)**, has been identified to provide additional monitoring for the study. This individual is a board-certified gastroenterologist in adult practice who is knowledgeable in the natural history and treatment of CD, but who is not directly involved in the study and has no conflict of interest, financial or otherwise. Following Penn IRB guidelines, all onsite, unanticipated SAEs will be reported to the Safety Officer promptly (within 3 working days) in the form of a written narrative. This will include a copy of the completed Serious Adverse Event form and any other information that will assist the understanding of the event. Significant new information for ongoing serious adverse events should be provided promptly to the study sponsor.

All other adverse events will be reported to the Safety Officer quarterly. Based on these reports, the Safety Officer will have the authority to suspend the study and to convene a Data and Safety Monitoring Board (DSMB) as necessary. We do not feel that a DSMB is necessary at the time of study launch given the open-label design and utilization of FDA-approved medications. This monitoring plan, in addition to screening for prolonged QTc and kidney and liver dysfunction, has been established to ensure the safety of the investigational regimen in this population.

9.5.1 Data and Safety Monitoring Plan

All data and records generated during this study will be kept confidential in accordance with Institutional policies and HIPAA regulations, and the Principal Investigator and other site personnel will not use such data and records for any purpose other than conducting the study. Safeguards are described under Data Management. The following groups of people at University of Pennsylvania may have access to this information: the research team, medical staff who are directly or indirectly involved in patients' care, and the Penn IRB. The data shared with the PennCHOP Microbiome Center will be coded. The results of this study may be shown at meetings or published in journals, but we will keep all identifying information private in any publication or presentation about this study.

10 Study Administration, Data Handling and Record Keeping

10.1 Confidentiality

Information about study participants 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 participant authorization informing the participant of the following:

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

In the event that a participant revokes authorization to collect or use PHI, the investigator, by regulation, retains the ability to use all information collected prior to the revocation of participant authorization. For participants 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 participant is alive) at the end of their scheduled study period.

10.2 Data Collection and Management

In order to ensure privacy, the collection of stool will be conducted in a private bathroom within the CTRC facility or the patient's home. Stool and swab samples will be coded, and the processing laboratory will receive samples labeled only with the participant ID number and date of collection. A master patient list with identifiers will be maintained by the PI and study team as a password-protected MS Excel spreadsheet saved on a secure shared research drive. Personal health information will be collected; however all data will be coded and kept in a secure online REDCap database, separated from identifiers. Paper records will be kept in a locked cabinet/desk in the study staff's office.

10.3 Records Retention

Study documents and data will be retained for at least two (2) years after the last participant has completed the study.

11 Study Monitoring, Auditing, and Inspecting

The investigator will permit study-related monitoring, audits, and inspections by the EC/IRB, the sponsor, 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.).

The Primary Investigator will monitor adverse events and unanticipated problems during the study to monitor ongoing participant safety. All SAE's will be reported to the safety officer within mandated time frames.

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

12 Ethical Considerations

This study is to be conducted in 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 independent Ethics Committee (EC) or Institutional Review Board (IRB), in agreement with local legal prescriptions, for formal approval of the study conduct. The decision of the EC/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 before commencement of this study.

12.1 Risks

There are potential risks to participants who agree to participate in this trial; however, we believe that the risks are relatively small and have been minimized by our design. For participants above the age of 65, any additional risk due to Ciprofloxacin should be mitigated through the additional precautions described in Section 3.1.2. The participants will have already been refractory to at least one conventional therapy. Many of these patients will otherwise be facing escalation of therapy to a medication that is considered experimental or will be facing surgery. We believe that the risks associated with this study are significantly less than the risks associated with surgery. The risks of each medication will be reviewed in detail as part of the consent process.

As the participants' concurrent therapies will not be discontinued and the intervention portion of this study lasts only two weeks, the probability of harm is very low. Participants can be withdrawn from the study at any time. Additionally, if a participants' condition worsens and a rescue therapy is deemed necessary by the study team or primary gastroenterologist, the participant will be withdrawn.

We believe that the therapeutic regimen described in this protocol is safe. These are medications that are already FDA approved for various indications. We are using standard dosing. Also, many of the medications in the proposed regimen are already utilized in children and adults with CD. Finally, in our preliminary data, we found a similar regimen to be safe in primates.

12.2 Benefits

The primary potential benefit is improvement in disease activity and potential salvaged response to the prior treatment regimen (immunomodulator or biologic medication). Additionally, in terms of indirect benefits, participation in this study will improve our understanding of the role of the gut microbiota in the inflammatory bowel diseases. The risks of participating in this study are outweighed by the potential benefits to participants who have active disease that is refractory to current therapy. Finally, the new knowledge about the gut microbiota that stands to be gained presents an additional benefit of this study, further contributing to the favorable balance of potential benefits to possible harms.

12.3 Risk Benefit Assessment

The risks of participating in this study are small, and these risks are outweighed by the potential benefits to participants who have very active disease that is refractory to current therapies. In addition, the new knowledge about the gut microbiota that stands to be gained presents a major benefit of this study, outweighs the risks, and justifies the conduct of this study.

12.4 Informed Consent Process / HIPAA Authorization

Information about study participants 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 participant authorization informing the participant of the following:

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

In the event that a participant revokes authorization to collect or use PHI, the investigator, by regulation, retains the ability to use all information collected prior to the revocation of participant authorization.
All data related to this trial will be recorded using the patients' assigned unique study number. Data will be reported only in a confidential manner such that the personal identity of any participant will not be identifiable. All study data will be maintained under a double locked system, such as a locked closet within a locked office or on a password protected computer in a locked office. Data will be entered into a secure REDCap database accessed on a password-protected computer.

13 Study Finances

13.1 Funding Source

This study is funded through a two-year grant from the Broad Medical Research Program at CCFA. The funds for microbiome analysis will come from the Joint Penn-CHOP Center for Digestive, Liver and Pancreatic Medicine.

13.2 Conflict of Interest

Any investigator 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 University of Pennsylvania investigators will follow the University conflict of interest policy.

13.3 Participants Stipends or Payments

Participants are not provided compensation for their participation in this study. However, in order to defray their parking and/ or travel costs, participants will receive $7 per in-person visit. This is equivalent to the amount required for parking in the PCAM parking garage for up to 3 hours (which is more than the length of each study visit) with validation from the PCAM information desk.

14 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 study sponsor. Any investigator involved with this study is obligated to provide the sponsor with complete test results and all data derived from the study.

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16 Appendix A: Adverse Reactions

16.1 Ciprofloxacin

Common reactions (>5%)	Occasional Reactions (1% to 5%)	Rare but Serious Reactions (<1%)
	Nausea, diarrhea, abnormal liver function tests, vomiting, rash	**Body:** headache, abdominal pain/discomfort, foot pain, pain, pain in extremities
		Cardiovascular: palpitation, atrial flutter, ventricular ectopy, syncope, hypertension, angina pectoris, myocardial infarction, cardiopulmonary arrest, cerebral thrombosis, phebitis, tachycardia, migraine, hypotension
		Central nervous system: restlessness, dizziness, lightheadedness, insomnia, nightmares, hallucinations, manic reaction, irritability, tremor, ataxia, convulsive seizures, grand mal convulsion, lethargy, drowsiness, weakness, malaise, anorexia, phobia, depersonalization, depression, paresthesia, abnormal gain
		Gastrointestinal: painful oral mucosa, oral candidiasis, dysphagia, intestinal perforation, gastrointestinal bleeding, cholestatic jaundice, hepatisis
		Hemic/Lymphatic: lymphadenopathy, petechia
		Metabolic/Nutritional: amylase increase, lipase increase, hyperglycemia, hypoglycemia
		Musculoskeletal*: arthalgia or back pain, joint stiffness, achiness, neck or chest pain, flare up of gout, muscle weakness
		Renal/Urogenital: interstitial nephritis, nephritis, renal failure, polyuria, urinary retention, urethral bleeding, vaginitis, acidosis, breast pain
		Respiratory: dyspnea, epitaxis, laryngeal or pulmonary edema, hiccough, hemoptysis, bronchospasm, pulmonary embolism

		Skin/Hypersensitivity: allergic reaction, pruritis, urticaria, photosensitivity/phototoxicity reaction, flushing, fever, chills, angioedema, edema of the face, neck, lips, conjunctivae or hands, cutaneous candidiasis, hyperpigmentation, erythema nodosum, sweating
		Senses: blurred vision, disturbed vision (changes in color perception, overbrightness of lights), decreased visual acuity, diplopia, eye pain, tinnitus, hearing loss, bad taste, chromatopsia

16.2 Vancomycin

Common Reactions (>5%)	Occasional Reactions (1% to 5%)	Rare but Serious Reactions (<1%)
Body: pyrexia, edema peripheral, fatigue	**Renal:** nephrotoxicity including renal failure, renal impairment, increased blood creatinine	**Ototoxicity:** hearing loss (associated with intravenous administration), vertigo, dizziness, tinnitus
Gastrointestinal: nausea, abdminal pain, vomiting, diarrhea, flatulence		**Hematopoetic:** reversible neutropenia (associated with intraveous administration), thrombocytopenia
Infections: urinary tract infection		**Miscellaneous:** anaphylaxis, drug fever, chills, nausea, eosinphilia, rash, Stevens-Johnson syndrome, toxic epidermal necolysis, vasculitis
Metabolism: hypokalemia		
Musculoskeletal: back pain		
Nervous system: headache		

16.3 Neomycin

Common Reactions (>5%)	Occasional Reactions (1% to 5%)	Rare but Serious Reactions (<1%)
		Neurological: ototoxicity, respiratory paralysis, neuromuscular blockage
		Renal: nephrotoxicity, including numbness, skin tingling, muscle twitching and convulsions
		Musculoskeletal: aggravation of muscle weakness *in patients with myasthenia gravis or parkinsonism*

		Miscellaneous: fungal overgrowth, malabsorption syndrome for various substances, including fat, nitrogen, cholesterol, carotene, glucose, xylose, lactose, sodium, calcium, cyanocobalamin and iron

16.4 Fluconazole

Common Reactions (>5%)	Occasional Reactions (1% to 5%)	Rare Reactions (<1%)
	Body: skin rash, headache	**Hepatic:** hepatotoxicity with no relation to daily dose, duration of therapy, age, or sex
	Gastrointestinal: nausea, vomiting, diarrhea, abdominal pain	**Immunological:** anaphylaxis, including angioedema, face edema and pruritus
		Cardiovascular: prolonged QT interval, torsade de pointes
		Central Nervous System: dizziness, seizures
		Hematopoietic and Lymphatic: leukopenia, including neutropenia and agranulocytosis, thrombocytopenia
		Metabolic: hypercholesterolemia, hypertriglyceridemia, hypokalemia
		Gastrointestinal: Cholestasis, dry mouth, hepatocellular damage, dyspepsia
		Musculoskeletal: myalgia
		Skin: acute generalized exanthematous-pustulosis, drug eruption, increased sweating, exfoliative skin disorders including Stevens-Johnson syndrome and toxic epidermal necrolysis, alopecia
		Miscellaneous: taste perversion, insomnia, paresthesia, somnolence, tremor, vertigo

16.5 Miralax (PEG 3350)

Common Reactions (>5%)	Occasional Reactions (1% to 5%)	Rare but Serious Reactions (<1%)
Nausea, abdominal fullness, bloating	Abdominal cramps, vomiting, anal irritation	**Immunological:** urticaria, rhinorrhea, dermatitis, anaphylaxis
		In patients > 60 years-old: upper GI bleeding from Mallory-Weiss tear, esophageal perforation, asystole, sudden dyspnea with pulmonary edema, "butterfly-like" infiltrates on chest X-ray after vomiting and aspirating PEG

		Miscellaneous: cardiac arrhythmia, tonic-clonic seizures and/or generalized loss of consciousness associated with electrolyte abnormalities

16.6 Promethazine

(frequencies are not defined by the literature)

Common Reactions	Rare but Serious Reactions
CNS: drowsiness/sedation and confusion (use caution with performing tasks requiring mental alertness such as operating machinery or driving)	**Cardiac:** cardiac conduction abnormalities; abnormal blood pressure (use with caution for people who have history of stroke, heart disease, and concurrent use of BP meds)
Derm: photosensitivity and skin pigmentation (slate gray), avoid prolonged sun exposure	**Neuro:** slow abnormal movements, increased rigidity, tongue protrusion, restlessness, mental status change, fever, muscle rigidity, autonomic instability, hallucinations, seizures
Misc: constipation, dry mouth, runny nose, blurred vision, urinary retention, temperature regulation	**Local:** injection site reactions (burning, edema, erythema, pain), abscess, local tissue destruction, clots
	Misc: cholestatic jaundice, hormone imbalance, abnormal platelets, increased risk for infection, loss of breathing (only seen in infants), allergic reaction

17 Appendix B: Investigational Drug Service Documentation

University of Pennsylvania - INVESTIGATIONAL DRUG SERVICE - Perelman School of Medicine

Main: 3600 Spruce St (Maloney Bldg, Ground Floor) | Satellite: 51 N. 39th St (1 Mutch Bldg) | Philadelphia, PA 19104

NOTE - THIS IS AN APPROXIMATION OF COSTS BASED ON CURRENT PRICES, NOT A 'CONTRACT' **Estimate #** 1508

Date Printed 7/21/2015

Protocol Title: **An Open-Label Pilot Study of Fundamental Modification of the Gut Microbiota in the Treatment of Refractory Crohn's Disease JULY 2015 VERSION 3** **REVISED**

Investigator: Albenberg, Lindsey, DO

Sponsor: Sponsor ID: pending

Subjects: Total of 20 outpatients to be enrolled

Detail/Narrative: For each subject, IDS would (a) purchase commercial vancomycin for injection and compound oral syringes, to administer as 500mg QID on Days 1-14), packaged as 14 'daily' sets of 4; (b) dispense a 4-day course of ciprofloxacin, packaged as individual 'AM' and 'PM' bottles together with the vancomycin syringes; c) dispense neomycin daily for 3 days; (d) prepare and dispense a single 8.3oz bottle of Miralax(R) ready to drink, along with one 20mg dose of bisacodyl; then (e) mail everything to the subject's home, 15 days prior to scheduled study visit 1 in CTRC. Quantities below are based on 20 completed courses plus enough overage for 2 additional courses (for loss, replacement, repeating missed doses, etc); if not needed then overall costs may be less. For FLUCONAZOLE, supplies & manufacturing assume enough for 11 active and 11 placebo courses, encapsulated for blinding purposes, based on a 400mg once/daily dose.

Actual enrollment may vary; as a service center the IDS applies user fees for dispensing or drug preparation at the time those expenses actually occur.

Activity	Cost	Units	Total
Study Initiation (procedures, product formulation, randomization scheme, planning, meetings, etc.)	$1,155.00	1	$1,155.00
Protocol And Inventory Maintenance (Per MONTH)	$25.50	18.00	$459.00
Materials:			
Manufacturing (Per 100 Caps): placebo fluconazole	$61.00	3.00	$183.00
Drug Purchase	$3.00	1.00	$3.00
Drug Purchase	$13.00	616.00	$8,008.00
Drug Purchase	$23.00	4.00	$92.00
Drug Purchase	$119.00	4.00	$476.00
Drug Purchase	$137.00	10.00	$1,370.00
Drug Purchase	$12.00	22.00	$264.00
Manufacturing (Per 100 Caps): blinded fluconazole	$61.00	3.00	$183.00
Preparation and Dispensation			
Prepare/dispense fluconazole (14 day course, 14 bottles); collect back used bottles to document compliance.	$29.55	20.00	$591.00
Prepare/dispense neomycin (3-day course, as 9 bottles); collect back used bottles to document compliance.	$20.85	20.00	$417.00
Prepare/dispense Miralax™ (8.3oz) with dosing cup and a 20mg dose of bisacodyl	$20.85	20.00	$417.00
Compound 1,120mL of oral vancomycin suspension (500mg/20mL concentration), then draw up into 56 individual oral syringes, hand-label and package into 14 separate ziploc bags (labeled as Day 1 through Day 14). Includes cost of suspending agents $(119/gallon), syringes ($92/100), labour, etc.	$184.25	20.00	$3,685.00
UPS shipping (estimated - note this is a pass-through expense)	$12.00	20.00	$240.00
Prepare/dispense ciprofloxacin (4-day course as 8 bottles)	$20.85	20.00	$417.00
Study Closure (copies, record archival, return or destroy supplies, etc.)	$122.00	1	$122.00

TOTAL assuming all subjects above complete treatment (note - for budget guidance only - actual enrollment may vary) **$18,082.00**

Per-subject cost (this is just an 'average' cost assuming a subject who completes the entire study) $335.70

Penn
University of Pennsylvania

Page 1 of 2

University of Pennsylvania - INVESTIGATIONAL DRUG SERVICE - Perelman School of Medicine

Main: 3600 Spruce St (Maloney Bldg, Ground Floor) | Satellite: 51 N. 39th St (1 Mutch Bldg) | Philadelphia, PA 19104

Study Initiation costs (everything that occurs BEFORE first enrollment - this cost occurs regardless of enrollment) ...	$1,155.00
DRUG PURCHASE COSTS (PASS-THROUGH EXPENSE) - subject to change, prices not guaranteed	$10,213.00

Additional Information:

Drug purchases are a pass-through expense at time of purchase, separate from user fees and prices are not guaranteed. Purchases listed above include vancomycin injection ($13/1gm vial x 616 vials), ciprofloxacin 750mg tabs ($23/btl of 50 x 4 btls), neomycin 500mg tabs ($119/100 x 4 btls), fluconazole 200mg tabs ($137/30 x 10 btls), Miralax® ($12 ea x 22 jars, 8.3oz each), bisacodyl 5mg tabs ($3/100 x 1 btl)

NOTE: Business manager should complete & return to IDS before start. Account# needed before ANY PURCHASES. If project not funded yet, an alternate account should be provided. This ESTIMATE is subject to change if actual IDS costs or sponsor requirements change. The IDS is a service center subject to OMB-A21 and required to adjust its fees periodically to reflect actual cost of services. Reduced monthly fees may apply for materials stored after close of a trial

Prepared by: Kenneth Rockwell Jr, PharmD, MS	Date: 4/15/2015	Business Admin. address and phone:
Business Admin. Signature	Date:	
Account Number (26-digits - call if you need to use a different format/system)		
Business Admin. E-Mail		
Business Admin. Embossment Below:	IRB #:	

18 Appendix C: CCFA-BMRP Sponsor Guidelines

BROAD MEDICAL RESEARCH PROGRAM AT CCFA
INFLAMMATORY BOWEL DISEASE GRANTS

The Crohn's & Colitis Foundation of America

CONTINUATION APPLICATION GUIDELINES AND INSTRUCTIONS

GENERAL INFORMATION

A primary purpose of BMRP-CCFA grants is to facilitate innovative IBD research to enable the Principal Investigator to obtain longer-term funding from other agencies. The crucial experiments that are necessary to validate the hypothesis should be done as early as possible in year one. If your hypothesis is confirmed by your preliminary data, we would expect you to apply for other longer-term funding during year one. Continuation support is possible if you made good progress and can document the need for a second year from the BMRP-CCFA in order to strengthen your chances for other funding.

The target deadline for a continuation application is **three months before** the end of the first funded period. It generally takes about two to three months to complete the peer review process and the application may need revisions. However, it is acceptable to submit the continuation application after the target deadline if more time is needed to demonstrate adequate progress. A no cost time extension of the project also is possible by written request. Please note that a late submission, even if funded, could result in a temporary lapse in funding.

If you receive a continuation award, we will extend the grant period, add the funding to the first year and change the due date for the final financial report. Our intent is that the funds for years one and two be combined so that you can continue to use any budgetary surplus from year one.

REVIEW PROCESS

A committee does not review proposals. Instead, leading investigators in each research area or topic write individual reviews and suggestions to improve the proposals. Reviews are conducted anonymously to encourage candid comments. So far, more than 2,787 reviewers from 45 countries have helped the BMRP-CCFA review proposals. The reviewers do not see each other's reviews and do not meet to forge a consensus report. Therefore, reviews can be different or even contradictory in some of the opinions or suggestions. The Principal Investigator receives excerpts from the reviewers' comments that could be helpful in revising or improving the proposal.

Grant and continuation application reviewers are required to sign confidentiality agreements prior to being sent the proposals in order to protect the investigators' ideas.

Reviewers' comments are advisory to the BMRP-CCFA. The final decision for continuation funding is made by the BMRP-CCFA. Funding for a second year is contingent upon demonstrated maximal progress in year one, validation of the original premise and clear need for additional preliminary data rather than completion of a project.

The reviewers of the continuation application might not be the same as those who reviewed the original grant application. We do provide a copy of the funded grant proposal to the continuation application reviewers.

Every attempt is made to ensure that there are no conflicts of interest between the investigators and their reviewers. Principal Investigators are encouraged to inform us of potential conflicts of interest so that we can avoid them in the review process.

You may suggest the names of potential reviewers. Please provide their complete mailing address, telephone number, fax number, e-mail address and area(s) of expertise.

If you are not funded, you may only submit a revised continuation application at our request. It will be up to you to decide what is an appropriate suggestion by a reviewer. Reviewers might not agree with each other or they could be incorrect. If you decide not to follow some of the reviewers' suggestions, you should explain your reasons clearly in a separate "Response to the Reviewers" document. Keep in mind that we might not use the same reviewers who evaluated your previous submission. Should you have any questions or concerns regarding the peer reviewers' comments, we encourage you to contact us.

Continuation application reviewers are asked to evaluate the following areas:

a. Evaluate achievements during the currently funded year:

   (i) Has the progress to date been reasonable?
   (ii) Has the initial hypothesis been substantiated by the findings?
   (iii) Will the findings create significant progress in IBD research and clinical practice?

b. Evaluate the plan for the next year:

   (i) Is a second year of funding needed in order to obtain longer-term funding from other agencies?
   (ii) Is the proposed plan for the next year scientifically sound?
   (iii) How innovative is the next year's plan for IBD research?
   (iv) Is the proposed budget for the next year appropriate?
   (v) Will the proposed work improve the lives of patients with IBD?

c. Indicate suggestions for the applicant regarding:

   (i) The progress report.
   (ii) The experimental plan for the next year.

d. If this project was submitted for funding to other sources and evaluated during the currently funded year, would the proposal for an additional year of BMRP-CCFA funding remedy the other agency's criticisms and make this project more competitive for national funding?

GENERAL APPLICATION GUIDELINES

1) The font used must be easy to read. The preferred fonts are either Arial 11 point or Times New Roman 12 point.

2) Except for the Continuation Application Face Sheet form, all pages must have the Principal Investigator's name typed in the upper right corner, formatted as last name, first name and middle initial or name.

PROPOSAL

Provide the information requested below in the order indicated. There are no page limitations, but be brief and concise! Paginate items 1 – 6 consecutively.

1) CONTINUATION APPLICATION SIGNATURE PAGE

Complete the Continuation Application Signature Page provided.

2) EXPENDITURES IN CURRENT YEAR *(Form Page 2)*

a) The first two columns of the form have been completed by the BMRP-CCFA to specify the currently funded budget.

b) Your accounting department must complete and certify the third column (expenditures to date) to reflect actual fiscal activity to date on your proposal.

c) The fourth column should be completed by you to indicate what is the remainder anticipated to be spent during the rest of the currently funded period.

d) Any estimated balance at the end of the currently funded year should be indicated in the fifth column (column 2 minus columns 3 + 4).

3) BUDGET FOR THE COMING YEAR *(Form Page 3)*

a) List the detailed budget items for all categories in column one.

b) Costs to attend the Annual BMRP-CCFA Investigator Meeting must be budgeted, either using funds remaining in year one or by requesting additional funds. The BMRP-CCFA no longer allows travel costs to attend scientific meetings other than the Annual BMRP-CCFA Investigator Meeting. Other travel necessary for performance of the project is allowable, but must be fully justified. Only economy airfare is allowable.

c) The second column should contain the amounts from the fifth column of page 2 **only if there are anticipated leftover funds from year one**. **Redistribute** the funds amongst the categories to reflect your actual need in the coming year. Do **not** show any deficits in this column. Do **not** complete this column unless there are funds remaining from year one.

d) Indicate your request for additional funds for the coming year in the third column.

e) The fourth column is leftover funds from year one + additional funds requested (columns 2 + 3).

4) **BUDGET JUSTIFICATION**

Provide a detailed justification of the budget for the coming year. If the budget is different than previously requested for this period, please explain and justify.

5) **PROGRESS REPORT** ***(All sections must be addressed and each question must be answered.)***

a) Progress –

i) Describe the major achievements for this project to date.
ii) If there have been no major achievements, explain why.
iii) Describe any negative results or failed experiments during the period.

b) What is the significance of the achievements to inflammatory bowel disease (IBD) diagnosis, therapy or prevention?

c) Research Plan Changes –

i) Did you modify the research plan? If so, why and in what way?
ii) Were there any unexpected directions or changes in the project based on the data generated so far?
iii) Would you have done your experiments any differently knowing what you know now? Explain.

d) Have there been any changes to the investigators originally slated to work on this project? If so, please explain.

e) Indicate whether your study involves human subjects or animals and complete the following information:

i) the number of subjects originally described to be studied in the currently funded year;
ii) the number studied so far in the currently funded year;
iii) the number to be completed during the balance of the currently funded year; and
iv) if the number of human or animal subjects to be completed during this currently funded year is either less or more than originally projected, explain why.

f) List all publications, abstracts and presentations resulting from this grant. If you have not yet provided a copy of this material to the BMRP-CCFA, attach one copy to this application.

g) Have you applied to other funding agencies to continue to work on this project?

i) If yes, please provide the following information:

(1) Name of the funding agency.

(2) Funding status (e.g., pending, received, or not funded).
(3) Type of grant requested (e.g., research grant, fellowship, career award).
(4) A copy of the peer review evaluations received.
(5) If funded by the other agency, please also include:
(a) Total amount awarded (direct plus indirect) for the entire project period.
(b) Number of years of support received.
(c) Year in which funding began.

ii) If you have ***not*** applied to other funding agencies to continue to work on this project, why not? When do you anticipate applying to other agencies for continued funding of this project? Which agencies?

iii) How will an additional year of funding from the BMRP-CCFA prepare you for applying elsewhere for support of this project?

h) Disclose and describe any patents resulting in whole or in part from the BMRP-CCFA supported project.

6) EXPERIMENTAL PLAN FOR THE NEXT YEAR *(All sections must be addressed)*

a) Describe and justify the work you would like to accomplish in the coming year:

i) why do you need additional preliminary data to apply for future funding of the project?
ii) specific aims;
iii) methodology;
iv) analysis;
v) anticipated outcomes; and
vi) relevance to IBD diagnosis, prevention and treatment.

b) If your study involves human or animal subjects, indicate the number anticipated to be studied in the coming year.

7) If this is a revised Continuation Application you must include a **RESPONSE TO REVIEWERS' COMMENTS**, this should be submitted as a separate document.

SUBMISSION INSTRUCTIONS

1) Attach one electronic signed copy into the Deliverables section of your BMRP-CCFA Grant Award and send an e-mail to me at the following address:

Michael Parks
Grants Manager
Broad Medical Research Program at CCFA
mparks@ccfa.org

2) Peer review will begin after receipt of the electronic signed application.

QUESTIONS AND ADDITIONAL INFORMATION

For questions, please contact:

Michael Parks, Grants Manager
E-mail: mparks@ccfa.org
Phone: 1-646-484-1695
Fax: 1-310-775-4067

mp 06/16/14

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