Title:	**A Randomized Pilot Study of Fundamental Modification of the Gut Microbiota in the Treatment of Refractory Crohn's Disease**
Short Title	Holiday
Drug or Device Name(s):	Polyethylene glycol, neomycin, vancomycin, ciprofloxacin, fluconazole
Sponsor:	The Broad Medical Research Program at CCFA, the National Institutes of Health National Institute of Diabetes and Digestive and Kidney Diseases
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TABLE OF CONTENTS

ABBREVIATIONS AND DEFINITIONS OF TERMS

AE	Adverse event
CBC	Complete blood count
CD	Crohn's disease
CDAI	Crohn's Disease Activity Index
CMP	Comprehensive metabolic panel
CMV	Cytomegalovirus
CRF	Case Report Form
CRP	C-reactive protein
CTRC	Clinical Translational Research Center
EKG	Electrocardiogram
ESR	Erythrocyte sedimentation rate
FCP	Fecal calprotectin
GI	Gastrointestinal
HBI	Harvey Bradshaw Index
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
TUNPRC	Tulane University National Primate Center
UC	Ulcerative Colitis

ABSTRACT

Context:

Recent evidence suggests that the gut microbiota is responsible for the stimulation of the intestinal immune system in Inflammatory Bowel Disease (IBD). 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.

Objectives:

The primary objective is to determine the effect of a novel gut microbiota-targeted therapeutic regimen in the management of active Crohn's Disease (CD) or IBDU that is refractory to conventional, immunosuppressive therapy. The regimen will deeply modify the gut microbiota and help to treat the gut inflammation associated with IBD, as well as rescue response to biologic or immunomodulator therapies.

Study Design:

This will be a randomized, placebo-controlled, double blind Phase 2a trial.

Setting/Participants:

The interventional arm (Group 1) of the study will recruit pediatric patients 6-18 years old with moderately active CD or IBDU who have had a loss of response to one or more biologic or immunomodulator therapies. Group 2 (observational arm) of the study will recruit patients aged 10 and older who are undergoing a GI endoscopy due to suspicion for active intestinal inflammation determined by physician global assessment (PGA).Study Interventions and Measures:

Only participants in the Group 1 of the study will undergo the study intervention. The study intervention includes a bowel lavage and oral antibiotics (3 days of vancomycin and neomycin followed by 11 days of vancomycin and ciprofloxacin) with or without an antifungal (fluconazole). Measures will be obtained for the evaluation of disease activity (PCDAI), fecal calprotectin, and C-reactive protein. Changes in the composition of the gut microbiota will also be assessed.Group 2 participants will not undergo the study intervention. Group 2 will be an observational group who are undergoing a bowel lavage as part of clinical care. Participants will provide stool samples before and after completing a PEG bowel lavage. Measures will be obtained for the evaluation of fecal calprotectin. Changes in the composition of the gut microbiota will also be assessed.

PROTOCOL SYNOPSIS

Study Title	Fundamental Modification of the Gut Microbiota in the Treatment of Refractory Crohn's Disease
Funder	The Broad Medical Research Program at CCFA, the National Institutes of Health, National Institute of Diabetes and Digestive and Kidney Diseases
Clinical Phase	Phase II
Study Rationale	The available evidence suggests that it is the commensal gut microbiota responsible for the stimulation of the intestinal immune system in the inflammatory bowel diseases. Thus, many IBD researchers and providers have questioned the current therapeutic approach. For instance, if the gut flora plays a role in the pathogenesis of IBD, then perhaps targeting the microbes rather than the immune system, or a combination of the two, would be more efficacious. Most IBD providers would agree that immune suppressive medications are the most reliable therapies that we have available right now, but that a therapeutic approach which targets both the gut microbes and the immune system is much more logical based on what we know regarding the pathogenesis of these diseases. We hypothesize that immune suppressive medications lead to persistent colonization with potentially pathogenic microbes that perpetuate disease chronicity in IBD patients. Additionally, we hypothesize that host immunosuppression could allow indolent colonizers of the colonic mucosa (symbionts) to behave as pathobionts, thereby causing progressive loss of response (LOR) to immune suppression. This study will evaluate the efficacy of a novel treatment regimen, employing non-immunosuppressive medications, in the management of refractory CD or IBDU. Refractory patients include those patients who have experienced LOR or primary nonresponse to an immunomodulator or a biologic. We will treat patients with a combination of gut microbiota-targeted therapies to

	restore a healthy gut microbiome composition. We believe that this strategy will both treat the gut inflammation associated with IBD as well as salvage response to immune suppressive therapies. In addition, the study will determine the effect of PEG lavage alone on fecal calprotectin and gut microbiota in patients with active CD or IBDU.
Study Objective(s)	**Primary** • 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 or IBDU that is refractory to conventional, immunosuppressive therapy. **Secondary** • 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 or IBDU refractory to immunosuppressive therapy. • To determine the relationship between the effectiveness of the proposed therapies with changes in gut microbiota composition. • To determine the effect of PEG lavage alone on the microbiome and the fecal calprotectin.
Study Design	Randomized, placebo-controlled, double blind Phase 2a trial
Subject Population **Key criteria for Inclusion and Exclusion:**	**Group 1-Intervention Participants** *Inclusion Criteria* 1. Parental/guardian permission (informed consent) and if appropriate, child assent.

2. Males or females 6-18 years of age
3. Current weight >10 kg (or 22 lb)
4. Ability to swallow pills
5. Normal kidney function
6. Normal AST, ALT, and alkaline phosphatase
7. Active CD or IBDU defined as PCDAI≥30
8. CRP ≥ 15mg/L (or 1.5mg/dL) or fecal calprotectin (FCP)>350mcg/g (within one month of enrollment)
9. 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

10. Girls who have menses and/or are ≥ 11 years of age must have a negative urine/serum pregnancy test and must use an acceptable method of contraception

Exclusion Criteria

11. Unwillingness to provide consent
12. Known allergy or intolerance to aminoglycosides or any of the medications used in this study, including medications within the same class
13. Current use of one of more of the following medications: 5-fluorouracil, digoxin, anticoagulants, theophylline, phenytoin, probenecid, duloxetine, clozapine, sildenafil, hydrochlorothiazide, cyclosporine, hypoglycemics,

terfenadine, tacrolimus, rifabutin, midazolam, and voriconazole

14. Known diagnosis of diabetes mellitus
15. Known or suspected stricturing disease producing obstructive symptoms
16. Active *Clostridium difficile* infection
17. Prolonged QTc interval as seen on enrollment EKG
18. Current use of antibiotics
19. Starting or increasing the dose of an IBD related medication within 4 weeks of screening
20. Participants who may be non-compliant with the study schedules or procedures

Group 2-Observational Arm Participants

Inclusion Criteria

1. Males or females 10 years of age and older.
2. Patients undergoing a clinical GI endoscopy due to suspicion for active intestinal inflammation determined by physician global assessment (PGA).
3. Undergoing a bowel preparation as part of clinical care.
4. Parental/guardian permission (informed consent) and if appropriate, child assent.

Exclusion Criteria

1. Antibiotic use within the past 30 days.
2. Current presence of an ostomy bag.
3. Patients undergoing a non- polyethylene glycol 3350 cleanout.
4. Unwillingness to provide informed consent.
5. Parents/guardians or subjects who, in the opinion of the Investigator, may be non-compliant with study schedules or procedures.

Number Of Subjects	60 pediatric participants will be enrolled with an estimated yield of 35 evaluable participants. Subjects will be enrolled from CHOP.
Study Duration	Group 1 subject participation will last 6-8 months; Group 2 subject participation could last up to two months. The entire study is expected to last 2 years.
Study Phases	*For Group 1 participants:* Screening Enrollment Treatment Phase Follow-Up
Efficacy Evaluations	Our estimates of efficacy will use a disease activity score (PCDAI) for clinical response (interventional patients only), FCP for mucosal inflammation, and CRP for systemic inflammation (for interventional patients only).
Safety Evaluations	*For Group 1 participants:* 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.
Statistical And Analytic Plan	The two arms of Group 1 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.
DATA AND SAFETY MONITORING PLAN	The PI is responsible for data quality management and ongoing assessment of safety.

TABLE 1: SCHEDULE OF STUDY PROCEDURES FOR INTERVENTIAL GROUP (GROUP 1)

	Screening/ Recruitment	Enrollment/ Day -17 - 0	Day 1	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	Study Drug Start	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	X
Web-based screening survey	X										
Daily Survey[a]		X		X	X	X	X	X	X[b]	X[b]	X[b]
Medical record review	X	X								X	X
Study Treatment			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[c]										
Rectal swabs for mucosal analysis	X	X[d]			X	X		X			
FCP	X[e]				X	X	X	X			
CBC (no diff)		X		Blood draw to store for future use	X	X		X			
CMP		X			X	X		X			
ESR		X			X	X		X			
CRP		X			X	X		X			
Serum Cystatin C		X									
Infliximab or adalimumab level[f]		X									
Vancomycin level[g]				X[g]	X[g]	X[g]					
PCDAI	X (within 1 month)	X		X	X	X		X			
EKG		X			X						

a. Online Redcap survey completed daily (Day-7 through Day 29).
b. Online Redcap survey completed once a week (Day 36 through 6 months).

c. C.diff will be performed if no record of negative C.diff within 1 month of enrolment.
d. Rectal swab samples will be collected once, either during screening or at the enrolment visit.
e. FCP will be performed if no record of test within 1 month of enrolment.
f. For participants on infliximab or adalimumab.
g. Serum vancomycin level will be tested as described in Section 5.1.5.

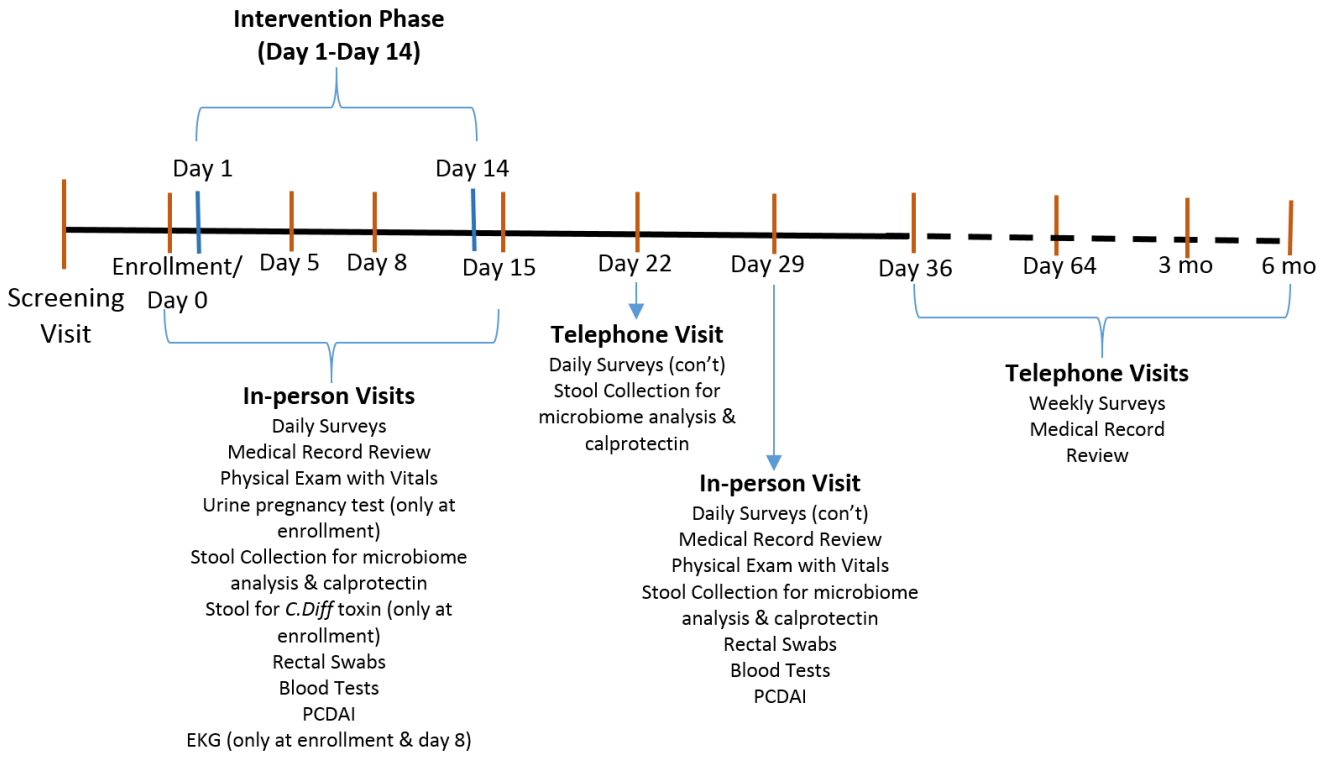
TABLE 2: SCHEDULE OF STUDY PROCEDURES FOR GROUP 2

Study Procedure Study Visit	Screening/ Recruitment (up to Day -1) Visit 0	Day -1 Day of Bowel Lavage*	Day 0 Day of Procedure*	Day 5 Post Procedure (+/- 24 hours) Visit 2	Day 12 Post Procedure (+/- 24 hours) Visit 3
Verbal Informed Consent	X				
Medical record review	X				
Distribution of study supplies	X			X	
Stool collection for microbiome analysis	X			X	X
Stool collection for FCP analysis	X**			X	X

*Clinical care procedures

**Not completed if participant has a FCP of ≥350 within 1 month of enrollment available in their clinical chart.

FIGURE 1: STUDY DIAGRAM, GROUP 1



1 BACKGROUND INFORMATION AND RATIONALE

1.1 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) or IBDU 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.

Additionally, this protocol will assess the effect of PEG lavage alone on the microbiome and the fecal calprotectin in patients with active IBDU or Crohn's disease.

1.2 Background and Relevant Literature

1.2.1 Inflammatory Bowel Disease: Current Therapies and Treatment Challenges

Inflammatory bowel disease (IBD), including CD, UC, and IBDU 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.

1.2.2 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]. It is unknown the effects of PEG lavage on the gut microbiome composition and FCP. It is expected that the effect of the PEG lavage on gut microbiota composition and fecal calprotectin will be comparable across patients who have been diagnosed with inflammatory bowel disease.

1.2.3 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.

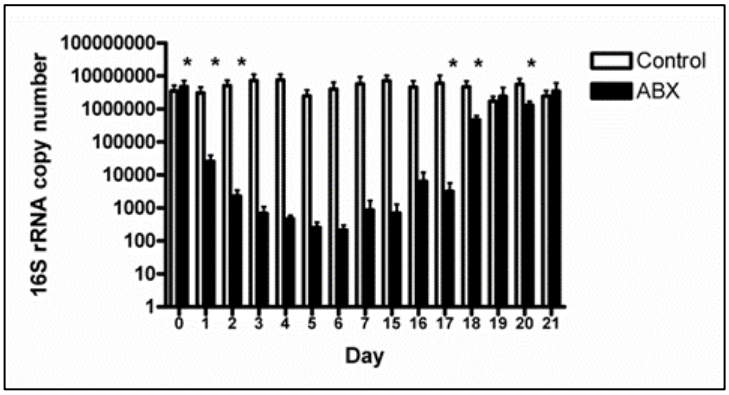


Figure 2: Time course of 16S rRNA gene copy number during oral antibiotic treatment (14 days of vancomycin plus neomycin) and upon discontinuing antibiotics on day 15 compared to control.

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, a retrospective study (CHOP IRB #15-011806) was performed to review CHOP's 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. In addition, 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.

Efficiency of Combination Antibiotic Therapy for Refractory IBD at PENN

Lindsey Albenberg, DO is performing a clinical trial in adults with a similar IRB-approved protocol at the University of Pennsylvania, which has shown potential for benefit and safety of the drug regimen. The primary endpoint in the Penn protocol is the change in disease activity, as measured by the Harvey Bradshaw Index (HBI) score, and FCP concentration, between the enrollment visit and Day 15. A reduction of HBI by 3 or more points is considered a clinically significant change in HBI.

Five adult participants have been enrolled. All five participants had lost response to anti-TNF alpha therapy and were not responding to either vedolizumab or ustekinumab. The first participant demonstrated a significant improvement in HBI that persisted to at least 2 months post antimicrobial therapy. This participant also had normalization of his fecal calprotectin from enrollment to day 15. The second two participants had decreases in the

HBI from enrollment to day 15. However, neither of these participants had a significant change in fecal calprotectin. One of these participants developed acute diarrhea on day 17. Stool infectious studies (including C. difficile) were negative. It was determined that the worsening of his diarrhea was related to progression of his already very severe CD. The fourth participant also demonstrated a significant improvement in HBI as well as normalization of the fecal calprotectin and this individual is still in the follow-up phase. The fifth participant is currently in the intervention phase, but this participant had an improvement in his HBI and normalization of his fecal calprotectin by day 8. Of note, there was one serious adverse event, but it was unrelated to the study procedures. There have been no other serious adverse events.

We are observing very significant reductions in fecal calprotectin from baseline to day 8 and day 15 in about half of the participants. We suspect that this represents healing of the mucosa in these very sick patients with IBD. However, given how robust the response has been (better response than expected), we want to be sure that the reduction in calprotectin truly represents healing of the tissue versus the PEG lavage flushing out the neutrophils. This will be examined in Group 2.

Table 2 shows change in HBI after completing the two week study regimen. These findings demonstrate the potential for benefit of the therapy proposed in this protocol.

TABLE 2: CHANGE IN HARVEY BRADSHAW INDEX (HBI) & DISEASE CATEGORY FOR HOLIDAY PARTICIPANTS AT PENN

Participant Number	HBI at Enrollment	HBI on Day 15	Number of Points Decreased	Clinically Significant (Y/N)*	Disease Category at Enrollment	Disease Category on Day 15
11001	11	3	8	Y	Moderate	Remission
11003	13	7	5	Y	Moderate	Mild
11005	9	5	4	Y	Moderate	Mild
11007	8	4	4	Y	Moderate	Remission
11011	7	5	2	N	Mild	Mild

*A reduction of HBI by 3 or more points is considered a clinically significant change in HBI.

Only one participant has shown an improvement in fecal calprotectin from baseline to day 15. However, it was a dramatic improvement. The n is still quite small.

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.

1.3 Selection of Drugs and Dosages

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 Children's Hospital of Philadelphia prior to colonoscopy and are also widely accepted in the literature [30]. In the animal study above (Figure 1) demonstrated ability to significantly reduce 16S rRNA gene copy number with a combination of two oral antibiotics – neomycin and vancomycin. This protocol has been adapted for human use because of the risk of ototoxicity, which is associated with long-term neomycin therapy. Thus, using neomycin only for the first three days, and then neomycin 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, the study will 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.

Animal studies have shown 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. This protocol chose standard pediatric dosing for the treatment of systemic or gastrointestinal infections (see Section 7.1 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 participate 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 steroid 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.

1.4 Compliance Statement

This study will be conducted in full accordance all applicable Children's Hospital of Philadelphia Research Policies and Procedures and all applicable Federal and state laws and regulations including 45 CFR 46, 21 CFR Parts 50, 54, 56, 312, 314 and 812 and the Good Clinical Practice: Consolidated Guideline approved by the International Conference on Harmonisation (ICH). All episodes of noncompliance will be documented.

The investigators will perform the study in accordance with this protocol, will obtain consent and assent, and will report unanticipated problems involving risks to subjects or others in accordance with The Children's Hospital of Philadelphia IRB 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 subjects during and after the study.

2 STUDY OBJECTIVES

The purpose of the study is to determine the efficacy of a novel treatment regimen, employing non-immunosuppressive medications, in the management of refractory CD or IBDU. Refractory patients include those patients who have experienced LOR or nonresponse to an immunomodulator or a biologic. LOR is defined as patients who were doing well initially on an appropriate dosage of an immunomodulator or a biologic and subsequently developed increased disease activity. We will treat patients with a combination of bowel lavage and oral antimicrobials. We hypothesize that this strategy will both treat the gut inflammation associated with IBD as well as salvage response to therapy.

2.1 Primary Objective (or Aim)

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 or IBDU that is refractory to conventional, immunosuppressive therapy.

2.2 Secondary Objectives (or Aim)

The secondary objectives are 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 or IBDU refractory to immunosuppression.

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

- To determine the effect of PEG lavage alone on the microbiome and the fecal calprotectin.

3 INVESTIGATIONAL PLAN

3.1 General Schema of Study Design

“Holiday” will be a randomized, placebo controlled, double blind Phase 2a trial. Participants will be enrolled into one of two groups. Group 1 will consist of pediatric CD or IBDU patients who have moderately active disease that is refractory to conventional, immunosuppressive therapy and will be receiving study intervention. Group 2 will consist of pediatric patients undergoing a clinically indicated PEG lavage in preparation for a clinically-indicated GI endoscopy and will not be receiving study intervention. We expect to enroll 60 participants to produce 20 evaluable participants in Group 1 and 15 evaluable participants in Group 2.A similar IRB-approved protocol is being conducted in adults at the University of Pennsylvania. The ultimate enrollment goal between both sites is 30 adults and 30 children (Group 1 only) to yield 20 evaluable participants in each group.

Group 1 recruitment will target patients who have demonstrated nonresponse or LOR to immunosuppressive therapy, and may be facing escalation of therapy or even surgery. Group 2 recruitment will target patients undergoing clinically indicated PEG lavage in preparation for a GI endoscopy.

Once enrolled, participants in Group 1 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 Pediatric Crohn's Disease Activity Index (PCDAI) and the Short Crohn's Disease Activity Index (sCDAI), the fecal calprotectin (FCP), a clinical marker of intestinal inflammation, and the C-reactive protein (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.

Participants in Group 2 will submit stool samples both before and after undergoing clinically indicated bowel lavage in preparation for a clinical GI endoscopy. The effect of the bowel lavage alone on disease activity (determined via fecal calprotectin levels) and composition of gut microbiota (determined via 16S gene qPCR, 18S gene qPCR, 16S gene sequencing, and ITS gene sequencing) will be assessed. 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 & Enrollment Phase

Potential participants will be identified through medical chart review, clinic schedules, inpatient lists, as well as physician referrals. Families will be contacted by a research team member and informed consent will be obtained. For prospective Group 1 participants, a REDCap Screening Questionnaire will be completed to confirm eligibility, and an enrollment visit will be scheduled. Participants will be given study supplies necessary for appropriate specimen collection. For prospective Group 2 subjects, consent will be obtained over the phone.

3.1.1.1 Group 1(Interventional Group)

For Group 1 participants, a verbal screening consent form will be obtained before collecting screening information. Once verbal consent is obtained, potential participants will be sent a REDCap Screening Questionnaire. Participants who seem to meet all inclusion criteria (see Section 3.4.1.1) will be scheduled for an enrollment visit and will be given study supplies. The screening consent will allow the subject to complete the screening questionnaire and provide samples at their first visit. Written informed consent, and assent when appropriate, will be obtained before undergoing any other study procedures. The study will be explained to each participant and their legal guardian when they come to the study site. If the subject and/or guardian agrees to participate, they will sign the written consent form.The enrollment visit will include a physical examination to determine eligibility based on clinical

parameters. Blood and stool samples will also be obtained at the enrollment visit for baseline values. If not completed clinically within 1 month of enrollment, the stool sample may be tested to rule out *Clostridium difficile* infection. If a participant tests positive for *C. difficile* 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. These participants can be re-screened once *C. difficile* has been treated and they have been tested negative for *C. difficile* infection. 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 who have menses and/or are $\geq$ 11 years old will have a urine pregnancy test. All participants will undergo electrocardiogram (EKG) to assess QTc interval at baseline. Participants and their parents 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.

3.1.1.2 Group 2 (Observational Group)

For Group 2 participants, a verbal informed consent form will be obtained before collecting a baseline stool sample and starting their clinically indicated bowel preparation. We will target patients who had a clinical disease activity score, physician global assessment, or laboratory values suggestive of ongoing active inflammation. If a clinical fecal calprotectin level greater than or equal to 350 mcg/g is not available within a month prior to enrollment, the baseline stool sample will be tested for fecal calprotectin level. . If the baseline fecal calprotectin is ≥350 mcg/g and if the patient's primary GI does not intend to start the patient on a corticosteroid or biologic within the 7 days following the procedure, subjects will then provide additional stool samples at time points 5 days and 12 days after their clinically-indicated procedure. These stool collection time points align with those of Group 1 participants, relative to their PEG lavage.

3.1.2 Study Treatment Phase

For Group 1 participants who are fully eligible, medications will be dispensed from The University of Pennsylvania's Investigational Drug Services (Penn IDS) and 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 1:1 in a double-blinded fashion to fluconazole or placebo for 14 days. See Section 7.1 for details about dose, frequency and duration of all study drugs.

On Day 2, participants will also undergo a bowel preparation with polyethylene glycol (PEG) 3350. 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 and/or their parent/guardian 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.

There is a +/- 2 day window within which the Day 15 visit must occur.

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

Group 1 participants who achieve clinical response (reduction of PCDAI by 15 or more points) or clinical remission (PCDAI <15) to the study intervention at Day 15, but have relapsed (PCDAI $\geq$ 30 or sCDAI $\geq$ 20) between days 30 and 64 (2 months), will be offered the option to repeat the 14 day study regimen. Once the study team is aware of a participant's disease relapse, the participant's symptoms will be observed for 2 weeks through the survey before starting their extension phase to confirm relapse. Participants who repeat the regimen will not require rescreening or a repeat enrollment visit. To reduce the risk of a prolonged QTc interval, the repeated regimen will exclude fluconazole/ placebo. The PEG lavage will also be excluded. An additional telephone visit (visit 6.1) will be conducted. This visit will take place at the end of the extension phase. 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, Study Schedule of Procedures). There is a +/- 3 day window for these two visits (Day 22 and 29). Finally, telephone follow-ups will occur at 3 months and 6 months following the intervention phase. There will be a 14-day window for the 3 month and 6 month follow-up telephone visits.

3.2 Allocation to Treatment Groups and Blinding

Within Group 1, participants will be randomized to one of two arms, one which will be receiving fluconazole in addition to antibiotics and one which will receive placebo. The randomization schedule will be 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.

3.3 Study Duration, Enrollment and Number of Sites

3.3.1 Duration of Study Participation

3.3.1.1 Group 1 Participants

The study duration per Group 1 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.

3.3.1.2 Group 2 Participants

The study duration per Group 2 participant could be up to 2 months.

3.3.2 Total Number of Study Sites/Total Number of Subjects Projected

The study will be performed at one site, The Children's Hospital of Philadelphia. A similar IRB-approved protocol is being conducted in adults at the University of Pennsylvania. The research will include outcome data from the Penn protocol. Data from these individuals will not be included in the enrollment numbers for this study and research activities under the Penn protocol will not be reported as part of the continuing review.

We expect to enroll 60 participants to produce 20 evaluable participants in Group 1 and 15 evaluable participants in Group 2.

3.4 Study Population

3.4.1 Group 1: Interventional Group; Participants who have had primary non-response or loss of response to conventional, immunosuppressive treatment

3.4.1.1 Inclusion Criteria —

1. Parental/guardian permission (informed consent) and if appropriate, child assent.
2. Males or females 6-18 years of age
3. Current weight >10 kg (or 22 lb)
4. Ability to swallow pills
5. Normal kidney function, defined by eGFR >90 mL/min/1.73m^3, estimated using serum cystatin C, creatinine, BUN, and height in the combined comprehensive eGFR equation.
6. Normal AST, ALT, and alkaline phosphatase, < 1.5 than the upper limit of normal for the reference range for the child's age, according to CHOP lab reference ranges.
7. Active CD or IBDU defined as PCDAI≥30

   **Patients who have a partial PCDAI of at least 20, including only the subjective reporting of symptoms over the past week (see Section 5.2.1), will be considered eligible for scheduling of an enrollment visit and will only be actively enrolled with a definite PCDAI score of 30 or above, inclusive of the physical examination and abdominal mass component.*
8. CRP >15mg/L (or 1.5mg/dL) or fecal calprotectin (FCP) > 350mcg/g (within one month of enrollment)
9. 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.*

10. Girls who have menses and/or are ≥ 11 years of age must have a negative urine/serum pregnancy test and must use an acceptable method of contraception, including abstinence, a barrier method (diaphragm or condom), Depo-Provera, or an oral contraceptive, for the duration of the study.

3.4.1.2 Exclusion Criteria —

1. Unwillingness to provide informed consent
2. Allergy or intolerance to aminoglycosides or any of the medications used in this study, including medications within the same class
3. Current use of one or more of the following medications: 5-fluorouracil, digoxin, anticoagulants, theophylline, phenytoin, probenecid, duloxetine, clozapine, sildenafil, hydrochlorothiazide, cyclosporine, hypoglycemics, terfenadine, tacrolimus, rifabutin, midazolam, and voriconazole
4. Known diagnosis of diabetes mellitus
5. Known or suspected stricturing disease producing obstructive symptoms
6. Active *Clostridium difficile* infection
7. Baseline QTc interval on EKG as follows:
    a. 3-5 yo: >412ms in males or >417ms in females
    b. 5-8 yo: >411ms in males or >409ms in females
    c. 8-12 yo: >407ms in males or >414ms in females
    d. >18 yo: > 430ms in males or > 450ms in females
8. Current use of antibiotics
9. Starting or increasing the dose of a IBD related medication within 4 weeks of screening including:
    e. Azathioprine or 6-mercaptopurine (6MP)
    f. Infliximab, adalimumab, certolizumab, or golimumab
    g. Natalizumab, vedolizumab, or ustekinumab

h. Methotrexate

i. Any 5-ASA compound (e.g. Lialda, Asacol, etc)

j. Prednisone, budesonide or other steroids delivered orally or rectally

10. Participants who, in the opinion of the investigator, may be non-compliant with study schedules or procedures

3.4.2 Group 2: Observational Group; Participants undergoing clinically indicated PEG lavage in preparation for a GI endoscopy.

3.4.2.1 Inclusion Criteria —

1. Males or females 10 years of age and older.
2. Patients undergoing a clinical GI endoscopy due to suspicion for active intestinal inflammation determined by physician global assessment (PGA).
3. Undergoing a bowel preparation as part of clinical care.
4. Parental/guardian permission (informed consent) and if appropriate, child assent.

3.4.2.2 Exclusion Criteria —

1. Antibiotic use within the past 30 days.
2. Current presence of an ostomy bag.
3. Patients undergoing a non- polyethylene glycol 3350 cleanout.
4. Unwillingness to provide informed consent.
5. Parents/guardians or subjects who, in the opinion of the Investigator, may be non-compliant with study schedules or procedures.

Subjects that do not meet all of the enrollment criteria may not be enrolled. Any violations of these criteria must be reported in accordance with IRB Policies and Procedures.

4 STUDY PROCEDURES

4.1 Group 1 Participants

4.1.1 Screening Visit

Screening visit will include:

- Informed consent
- Email or complete screening questionnaire
- Schedule enrollment visit
- Distribute study supplies

4.1.2 Enrollment Phase (Visit 1)

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

- Written informed consent & assent (if applicable)
- Review and documentation of eligibility criteria
- Obtain collected stool sample for analysis of microbiome
    - If no record of negative *C. difficile* toxin within 1 month of enrollment, stool will be used to test for *C. difficile* toxin
    - If no record of fecal calprotectin within 1 month of enrollment, stool will be used to test fecal calprotectin
- Vital signs
- Anthropometric measurements
- Physical exam, including CD disease symptom assessment for PCDAI
- Rectal swabs (2) for mucosally-associated microbiome analysis
- Blood sample (CBC, CMP, CRP, ESR, serum cystatin C, biologic levels if applicable with extra stored for future use)
- Urine pregnancy test for female participants (if applicable)
- EKG
- Medication education
- Distribution of Gatorade (and a pitcher)
- Distribution of supplies

4.1.3 Study Treatment 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. The study team will instruct the participant 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.

4.1.4 Daily Surveys

The participant and/or their parent/guardian will be asked to complete an online survey through REDCap asking a variety of questions to monitor disease activity. Participants and their parent/guardian will be prompted to identify any concerns including medication side effects and/or worsening of symptoms. This survey will be completed as listed in Table 1, Schedule of Study Procedures.

Survey responses will be monitored by study staff through REDCap on a regular basis. If surveys are not completed as scheduled, the coordinator may contact the participant to ensure compliance.

If a participant is enrolled in the study who does not have daily access to the internet and/or email, a daily survey paper diary will be provided to them. In order to ensure compliance for

this group, they will be required to ship the completed paper surveys to the study team on a weekly basis. Shipping labels will be provided in advance to enable this practice.

4.1.5 Study Visit 2 (Day 5)

The visit will include:

- Vital signs
- Anthropometric measurements
- Physical exam, including CD disease symptom history for PCDAI
- Blood sample (vancomycin level, with extra stored for future use to evaluate metabolomics and neomycin levels)
- Obtain stool sample
- Adverse event assessment (see Section 8)
- Study drug compliance assessment
- Update current medications log
- Daily Survey
- Distribution of supplies

4.1.6 Study Visit 3 (Day 8)

The visit will include:

- Vital signs
- Anthropometric measurements
- Physical exam, including CD disease symptom assessment for PCDAI
- EKG
- Rectal swab for mucosally-associated microbiome analysis
- Blood sample (CBC, CMP, CRP, ESR with extra stored for future use and vancomycin level, if applicable)
- Obtain stool sample
- Adverse event assessment (see Section 8)
- Study drug compliance assessment
- Update current medications log
- Daily Survey
- Distribution of supplies

4.1.7 Study Visit 4 (Day 15)

The visit will include:

- Vital signs
- Anthropometric measurements
- Physical exam, including CD disease symptom assessment for PCDAI
- Rectal swab for mucosally-associated microbiome analysis
- Blood sample (CBC, CMP, CRP, ESR with extra stored for future use and vancomycin level, if applicable)
- Collect stool sample

- Adverse event assessment (see Section 8)
- Study drug compliance assessment and collect any unused study drug
- Update current medications log
- Daily Survey
- Distribution of supplies

4.1.8 Follow-up Phase

Participants will be followed from Day 16 through 6 months. The study team will conduct a telephone call on Day 22 and an in-person visit on Day 29. From Day 30 through 6 months post-treatment, participants and/or their parent/guardian will continue to record symptoms weekly in REDCap. Telephone follow-up will occur at 3 months and 6 months.

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

A telephone follow-up will be conducted on Day 22 (+/- 3 days). During the telephone session, questions will be asked to assess the general state of the participant's IBD and their current medication regimen. The participant's electronic medical record will be reviewed. Any recent surgeries, hospitalizations, and *C difficile* test results since their last study visit will be recorded. Participants and their parent/guardian will be reminded to ship or bring in a stool sample.

4.1.8.2 Study Visit 6 (Day 29)

The visit will be conducted on Day 29 (+/- 3 days). The visit will include:

- Vital signs
- Anthropometric measurements
- Physical exam, including CD disease symptom assessment for PCDAI
- Rectal swab for mucosally-associated microbiome analysis
- Blood sample (CBC, CMP, CRP, ESR with extra stored for future use)
- Collect stool sample
- Adverse event assessment (see Section 8)
- Study drug compliance assessment
- Update current medications log
- Daily Survey

4.1.8.3 Telephone Follow-Ups: 3 Months & 6 Months

Telephone follow-up will also occur at 3 months and 6 months (+/- 2 weeks). Questions will be asked to assess the general state of the participant's IBD and their current medication regimen. The participant's electronic medical record will be reviewed. Any recent surgeries, hospitalizations, and *C difficile* test results since their last study visit will be recorded.

4.1.9 Concomitant Medication

All prior and concomitant medications used within 30 days prior to the screening visit and through the end of the study will be recorded. The dates of administration, dosage, and reason for use will be collected. All concomitant medications will be continued with the exception of probiotics and antibiotics which must be discontinued prior to the start of the

intervention phase (day 1-14). If potential participants are on prescribed antibiotics related to their IBD, they will be required to stop those antibiotics in order to participate in this study. If potential participants are on antibiotics for non-IBD related reasons, they will be required to complete their current course before enrolling in this study. Participants already on steroids as part of their IBD regimen will be required to maintain their dose and regimen during the 14 days (Days 1-14) of the study drug intervention. If steroid 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.

4.1.10 Rescue Medication Administration

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 on whether or not to withdraw the participant from the study. If the participant receives rescue medication(s) during Days 1-14 of the study, the study drugs will be stopped and they will be considered a non-responder. Participants will be followed until the end of the 6 month time point of the study schedule.

4.2 Group 2 Participants

4.2.1 Visit 1: Baseline Sample Prior to bowel cleanout

- Obtain informed consent
- Medical Record Review
- Distribution of study supplies
- Collect stool sample

4.2.2 Visit 2: Day 5 (+/- 24 hours) after GI endoscopy

- Medical Record Review
- Collect stool sample
- Distribute supplies for next sample collection

4.2.3 Visit 3: Day 12 (+/- 24 hours) after GI endoscopy

- Medical Record Review
- Collect stool sample

4.3 Subject Completion/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 PI 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. If a subject withdraws from the study all research samples and data collected prior to withdraw will remain part of the study. It will be documented whether or

not each participant completes the clinical study. If the PI 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 and to the IRB (when applicable).

4.3.1 Early Termination Study Visit

Group 1 participants who withdraw from the study will have all procedures enumerated for the last visit as the early termination visit. Participants who are withdrawn during the intervention phase will be asked to return the investigational product.

5 STUDY EVALUATIONS AND MEASUREMENTS

5.1 Screening and Monitoring Evaluations and Measurements

5.1.1 Group 1

5.1.1.1 Medical Record Review

Include a listing of the variables that will be abstracted from the medical chart (paper or electronic).

- Date of birth
- MRN
- Mailing address
- Email address
- Telephone number
- Sex
- Race
- Height
- Weight
- IBD 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
- Interview with participant (such as bowel frequency, abdominal pain, general well-being)
- Physical examination
- Laboratory tests (including ESR, CRP, albumin, hematocrit, hemoglobin, fecal calprotectin, stool C. difficile toxin, drug levels for biologics)

5.1.1.2 Physical Examination

A physical examination will be performed in order to calculate the PCDAI at all in-person study visits for Group 1 participants. A study investigator will document whether there is an abdominal mass (0=no tenderness, no mass, 5= tenderness, no mass without tenderness, 10 = tenderness, involuntary guarding, definite mass). A study investigator will also document any extraintestinal manifestations of disease, including arthralgia, uveitis, erythema nodosum, aphthous ulcers, *Pyoderma gangrenosum*, anal fissure, new fistula, and/or abscess. Documentation may be completed in EPIC.

5.1.1.3 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 as listed in Table 1, Schedule of Procedures.

5.1.1.4 EKG

An EKG will be performed by trained 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. Prolonged QTc intervals are defined in section 3.4.2. 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.

5.1.1.5 Laboratory Blood Evaluations

Blood will be collected and processed by trained staff. The following testing will be performed:

- **ESR**

  ESR will be evaluated at the enrollment visit, day 8, day 15, and day 29.

- **CRP**

  CRP will be evaluated at the enrollment visit, day 8, day 15, and day 29.

- **CMP**

  CMP will be evaluated at the enrollment visit, day 8, day 15, and day 29.

- **CBC without differential**

  CBC without differential will be evaluated at the enrollment visit, day 8, day 15, and day 29.

- **Serum cystatin C**

  Serum cystatin C will be evaluated at the enrollment visit. Serum cystatin C, creatinine, BUN, and height will be used in the combined comprehensive eGFR equation to estimate eGFR in order to confirm eligibility.

- **Vancomycin Level**

  Serum vancomycin levels will be tested on day 5 to monitor for systemic absorption of vancomycin. If the result is above the therapeutic range, vancomycin will be discontinued and serum vancomycin will be tested on day 8. If on day 8, serum vancomycin level is below the therapeutic range, vancomycin will be restarted, but the dose will be decreased by 25%. If the serum vancomycin level remains therapeutic or above therapeutic, vancomycin will not be restarted and the serum level will be rechecked on day 15.

- **Drug levels for participants on biologics stored 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.

- **Extra blood for storage for future testing**

  Extra 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.

Total blood draws will not exceed 3ml/kg or 50 ml (whichever is greater) within an 8-week period.

5.1.1.6 Pregnancy Testing

A urine pregnancy test will be performed for female subjects ≥ 11 years of age and girls <11 years who are physically capable of becoming pregnant.

5.1.1.7 Stool Collection, Shipping, and Analysis

Each participant will be provided with stool collection kits to take home during the course of the study. 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 will be returned to CHOP using pre-paid mailers, by dropping it off at the main hospital or a satellite location, or by submitting it to a study team member at a study visit.

All specimens remaining after the study is complete will be retained for possible future use or it is deemed by the investigators that the specimens are no longer needed.

Stool sample from the enrollment will be sent to a CHOP lab for *C. difficile* testing (if not tested clinically within one month of enrollment).

Stool samples will be aliquoted for FCP testing and microbiome analyses. The remaining Stool will be stored in the laboratory of Dr. Gary Wu at the University of Pennsylvania. Microbiome analysis will occur through the PennCHOP Microbiome Center.

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. Swabs will stored in a -80 freezer at the University of Pennsylvania.

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

5.1.2 Group 2

5.1.2.1 Medical Record Review

- Date of birth
- MRN
- Mailing address
- Email address
- Telephone number
- Sex
- IBD history (including date of diagnosis, and disease location)
- Prior use of medications
- Current medications
- Procedure reports and phone encounters associated with bowl prep
- Physical examination
- Laboratory tests (including ESR, CRP, albumin, hematocrit, hemoglobin, fecal calprotectin, stool C. difficile toxin, drug levels for biologics)

The above data elements will be abstracted manually from the electronic medical record by study staff.

5.1.2.2 Sample Collection

Stool samples will be collected at specified time points during the study, before and after undergoing PEG lavage. If participants are not seen in clinic during the study timeframe, specimen collection direction and supplies will be mailed to the participant. The family will use a stool collection hat to obtain the stool. Samples will be returned to CHOP. Samples will be processed for microbiome analysis identically to those of Group 1 participants.

5.2 Efficacy Evaluations

5.2.1 Diagnostic Tests, Scales, Measures, etc.

The Pediatric Crohn's Disease Activity Index is a validated, non-invasive scale that is widely used in clinical trials to assess Crohn's disease activity among children [31]. The PCDAI scale incorporates self-reported symptoms, historical features, laboratory data (hematocrit, albumin, and ESR), and physical examination findings for an overall score of 0 to 100 [31]. The PCDAI includes the following measurements: 1. Subjective reporting of the degree of abdominal pain, stool pattern, and general well-being (recall over the past week). 2. Presence of extraintestinal manifestations, such as fever, arthritis, rash, and uveitis. 3. Physical examination findings. 4. Weight change and either height change or height velocity. 5. Hematocrit (HCT), erythrocyte sedimentation rate (ESR), and serum albumin.

A partial PCDAI score will be used to evaluate patients at enrollment. This will consist of the PCDAI score without the physical exam or laboratory component (HCT, ESR, and albumin) of the assessment. The subject reports the degree of abdominal pain, stools per day, and general well-being over the previous week. The scoring system is outlined in Table 3.The partial PCDAI is calculated as the sum of the scores from the 3 subjective questions in

the PUCAI, and ranges from 0 to 30. The partial PCDAI includes the following three measurements; scoring for each measurement is indicated in parentheses:

Table 3: Partial PCDAI Score Criteria

Symptom	Degree	Score
Abdominal Pain	None	0
	Mild: Brief, does not interfere with activities	5
	Moderate/Severe: Daily, longer lasting, affects activities, nocturnal	10
Stools (per day)	0-1 liquid stools, no blood	0
	Up to 2 semi-formed with small blood, or 2-5 liquid	5
	Gross bleeding, or ≥6 liquid, gross blood, or nocturnal diarrhea	10
Patient Functioning, General well-being	No limitation of activities, well	0
	Occasional difficulty in maintaining age-appropriate activities, below par	5
	Frequent limitation of activity, very poor	10

Active disease will be defined as a PCDAI score >10 regardless of the FCP concentration [43]. To be defined as having achieved a full clinical response (remission), patients must have a PCDAI ≤10 or a 15 point reduction from week 0, and a FCP concentration ≤200 mcg/g. Group 1 participants with PCDAI≤10 or a 15 point reduction from week 0 but with elevated FCP (i.e. >200 mcg/g) will be considered partial responders. The sCDAI, a modified score validated in adults that includes only the history elements[34], will be administered through the daily REDCap surveys on Day 1-29 and then weekly thereafter (see Section 4.3.1). The sCDAI is validated in the adult population, but there is no equivalent measure in the pediatric population. Therefore, the sCDAI will be used to evaluate disease activity in daily surveys.
Both PCDAI and sCDAI are validated measures of CD disease activity. The sCDAI is validated only in the adult population, but there is no equivalent measure in the pediatric population. Therefore, the sCDAI will be used to evaluate disease activity in daily surveys.

5.2.2 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 [35]. 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 [41]. 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 [42].

In this study, FCP concentration will be assessed using ELISA methods. The results of each stool calprotectin will be entered into CHOP's electronic medical record and shared with the participant's primary gastroenterologist. Stool calprotectin is used as a marker of intestinal inflammation. It is standard-of-care at the CHOP Inflammatory Bowel Center to consider any stool calprotectin value above 250μg/g as significantly elevated.

5.2.3 C-reactive protein (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. CRP will be tested through a CHOP laboratory.

5.2.4 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 the preliminary data, samples may be analyzed 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].

5.3 Safety Evaluation

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 8). Adverse events will be compared to all known side effects of the antimicrobials prescribed through the current study (see Section 9.5.2). All adverse events will be tracked and assessed by the PI. Adverse events will be reported according to CHOP Research Policy and Procedure – please see Section 9.5.1 for additional safety monitoring details for this study.

6 STATISTICAL CONSIDERATIONS

6.1 Primary Endpoint

The primary endpoint will be the change in disease activity, as measured by PCDAI and FCP concentration, between the enrollment visit and Day 15 for Group 1 participants. All participants who withdraw for any reason prior to day 15 will be considered treatment failures.

To be defined as having achieved a full clinical response (remission), patients must have:

- A PCDAI ≤10 or a 15-point reduction from week 0, and a FCP concentration ≤200 mcg/g.
- Participants with PCDAI≤10 or a 15-point reduction from enrollment but with elevated FCP (i.e. > 200 mcg/g) will be considered partial responders.

6.2 Secondary Endpoints

Secondary endpoints will include the following:

- The change in CRP 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 or IBDU that is 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).
- The change in FCP between baseline, Day 5 after procedure, and Day 12 after procedure in Group 2 participants.

6.3 Statistical Methods

The two treatment arms in Group 1 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 CRP. For clinical response (reduction of score on PCDAI by 15 or more points) we will report the proportion and binomial 95% confidence intervals. Similar methods will be used for remission (PCDAI ≤10). 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 CRP. Therefore, we will use a paired t-test (after applying a log transformation if necessary) to establish whether the CRP 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 [44].

We are not examining efficacy in Group 2. The primary outcome is reduction in FCP with PEG lavage. We will use a paired t-test (after applying a log transformation if necessary) to establish whether the FCP concentration is lower following PEG lavage than at baseline. From our preliminary data and the expected mean and standard deviation of the paired differences, 15 patients will allow us to achieve 80% power and a level of significance of 5%.

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 the preliminary data, it is 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 (PCDAI, FCP, and CRP). 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.

The 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 [47, 48], 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.

6.3.1 Safety Analysis

All subjects enrolled into the study will be included in the safety analysis. The frequencies of AEs by type, 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.

7 STUDY MEDICATION

7.1 Intervention Description and Regimen

Only Group 1 participants will be administered the study drug regimen. The antimicrobial regimen will include 3 days of vancomycin and neomycin followed by 11 days of vancomycin and ciprofloxacin. Group 1 participants will also be randomized in a double-blinded fashion to fluconazole or placebo for 14 days.

Drug	Dosage Form	Dosage	Regimen
Vancomycin	500 mg/ 20 mL oral suspension	40 mg/kg/day in 4 divided doses, rounded to the nearest 50mg; not to exceed 500 mg PO every 6 hours	Days 1-14
Neomycin	500 mg tablet	10-20 kg: 250 mg PO every 8 hours 20-40 kg: 500 mg PO every 8 hours >40 kg: 1000 mg PO every 8 hours	Day 1-3
Ciprofloxacin	250 mg tablet, 500 mg tablet, 750 mg tablet	10-12.5 kg: 125 mg PO twice per day 12.5-22.5 kg: 250 mg PO twice per day 22.5-27.5 kg: 375 mg PO twice per day 27.5-37.5 kg: 500 mg PO twice per day 27.5-40 kg: 625 mg PO twice per day >40 kg:750 mg PO twice per day	Days 4-14
Encapsulated fluconazole or placebo capsule	50 mg capsule, 100 mg capsule	10-20 kg: 50 mg PO in one dose daily 20-25 kg: 100 mg PO in one dose daily 25-30 kg: 150 mg PO in one dose daily 30-40 kg: 200 mg PO in one dose daily 40-50 kg: 250 mg PO in one dose daily 50-60 kg: 300 mg PO in one dose daily >60 kg: 400 mg PO in one dose daily	Days 1-14
Miralax (PEG 3350)	Powder (dissolved in Gatorade or Crystal Lite)	10-13 kg: 51 g dissolved in 24 oz 14-17 kg: 68 g dissolved in 32 oz 18-22 kg: 85 g dissolved in 40 oz 23-26 kg: 102 g dissolved in 48 oz 27-30 kg: 119 g dissolved in 56 oz 31-34 kg: 136 g dissolved in 64 oz 35-40 kg: 153 g dissolved in 64 oz 41-42 kg: 170 g dissolved in 64 oz 43-46 kg: 187 g dissolved in 64 oz 47-50 kg: 204 g dissolved in 64 oz >50 kg: 238 g dissolved in 64 oz	Day 2

7.1.1 Dispensing

The University of Pennsylvania's Investigational Drug Service (IDS) will prepare and dispense the drugs for this study.

7.1.2 Receipt

After confirmation of eligibility, participants will be sent the antimicrobial and lavage regimen listed above directly from Penn IDS to their home mailing address. Participants and their parent/guardian 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.

7.1.3 Storage

Study medications will be prepared, stored, and dispensed by Penn IDS as described in the investigational brochures.

7.1.4 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 clearly, with each dose separated into blisters. The vancomycin suspensions will be packaged in separate Ziploc bags per day. Each bag will contain four syringes containing the appropriate dosage of vancomycin. The Day 2 bag will additionally contain a bottle with PEG 3350. At the enrollment visit, participants will be provided with a 64 ounce water pitcher plus either 2 32 ounce bottles of Gatorade.

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 the prescribed total dosage for the oral suspension (500 mg/20 mL concentration) drawn up into individual syringes based on dosage. For fluconazole, IDS will encapsulate the original formulation tablets so that they will look identical to placebo capsules.

7.1.5 Administration and Accountability

Participants will be provided with all study medications by tracked courier to their preferred address following the enrollment visit and consequent confirmation of their eligibility. Participants will take all medications orally. All unused study medications and empty containers will be collected from each participant and/or their parent/guardian at the completion of their participation.

7.1.6 Participant Compliance Monitoring

Compliance will be monitored through study staff observation of empty blister packs and/or syringes at each in person visit (see Section 7.1.7). At each in-person visit, participants and/or their parent/guardian 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 and/or their parent/guardian throughout the study.

7.1.7 Return or Destruction of Investigational Product

Adequate records of study drug receipt and disposition will be maintained by the University of Pennsylvania Investigational Drug Services. The purpose of these records is to ensure regulatory authorities that the investigational new drug will not be distributed to any person who is not a study subject under the terms and conditions set forth in this protocol. The study medication is to be prescribed by the Investigator or designee 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 the study team.

8 SAFETY MANAGEMENT

8.1 Clinical Adverse Events

Clinical adverse events (AEs) will be monitored throughout the study.

8.2 Adverse Event Reporting

Unanticipated problems related to the research involving risks to subjects or others that occur during the course of this study (including SAEs) will be reported to the IRB in accordance with CHOP IRB SOP 408: Unanticipated Problems Involving Risks to Subjects. AEs that are not serious but that are notable and could involve risks to subjects will be summarized in narrative or other format and submitted to the IRB at the time of continuing review.

8.3 Definition of an Adverse Event

An adverse event is any untoward medical occurrence in a subject who has received an intervention (drug, biologic, or other intervention). The occurrence does not necessarily have to have a causal relationship with the treatment. An AE can therefore be any unfavorable or unintended sign (including an abnormal laboratory finding, for example), symptom, or disease temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product.

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.

8.4 Definition of a Serious Adverse Event (SAE)

An SAE is any adverse drug experience occurring at any dose that results in any of the following outcomes:

- death,
- a life-threatening event (at risk of death at the time of the event),
- requires inpatient hospitalization or prolongation of existing hospitalization,
- a persistent or significant disability/incapacity, or

- a congenital anomaly/birth defect in the offspring of a subject.

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 subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

8.4.1 Relationship of SAE to study drug or other intervention

The relationship of each SAE to the study intervention should be characterized using one of the following terms in accordance with CHOP IRB Guidelines: definitely, probably, possibly, unlikely or unrelated.

8.5 IRB/IEC Notification of SAEs and Other Unanticipated Problems

The Investigator will promptly notify the IRB of all on-site unanticipated, serious Adverse Events that are related to the research activity. Other unanticipated problems related to the research involving risk to subjects or others will also be reported promptly. Written reports will be filed using the eIRB system and in accordance with the timeline below.

Type of Unanticipated Problem	Initial Notification (Phone, Email, Fax)	Written Report
Internal (on-site) SAEs Death or Life Threatening	24 hours	Within 2 calendar days
Internal (on-site) SAEs All other SAEs	7 days	Within 7 business days
Unanticipated Problems Related to Research	7 days	Within 7 business days
All other AEs	N/A	Brief Summary of important AEs may be reported at time of continuing review

8.5.1 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) will be submitted to the IRB. The investigator is responsible for ensuring that all SAE are followed until either resolved or stable.

8.6 Medical Emergencies

If any medical emergencies occur during the course of the study, patients will be treated for said medical emergency using standard hospital medical procedures. One such medical emergency that may occur is a severe allergic reaction to a study medication.

9 STUDY ADMINISTRATION

9.1 Treatment Assignment Methods

9.1.1 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).

9.1.2 Blinding

The randomization schedule will be 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.

9.1.3 Unblinding

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 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 study team.

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.

9.2 Data Collection and Management

- In order to ensure confidentiality, all subjects will be given a study ID number that will used to identify them on all data collection documents. Data will also be recorded on a shared REDCap account, an online database shared amongst the study team members at CHOP and Penn. All information collected in this study will be kept confidential as required by law.

- An enrollment log linking PHI to study ID number will be kept on a password protected excel document on the CHOP Research Secure Server (SAND). Only study personnel at the CHOP site will have access to the enrollment log. The study team will be responsible for data collection, data management and accurate record keeping. Upon completion of all study procedures and analyses, the enrollment log, linking PHI to subject study IDs will be destroyed when deemed no longer necessary.

- The study team will oversee appropriate collection, storage and shipping of samples. For stool samples collected during post discharge follow ups, subjects will be asked to either bring samples to CHOP or ship the samples to the laboratory using provided pre-paid shippers. Samples will be processed and stored in a -80°C freezer. Subject will be instructed to properly label the samples with the date of collection, study ID

and visit number. Subjects will be reminded not to include any identifying information on samples. The PennCHOP Microbiome Program will receive the samples with study IDs, visit number and dates of collection. The lab will not receive any identifying information.

9.3 Confidentiality

All data and records generated during this study will be kept confidential in accordance with Institutional policies and HIPAA on subject privacy. The Investigator and other site personnel will not use such data and records for any purpose other than conducting the study. Data will not be reused or disclosed to any other person or entity, except as required by law or for authorized oversight of the research project. Safeguards to maintain subject confidentiality are described in the above section on data collection and management. The following groups of people at CHOP may have access to this information: the research team, Penn IDS, medical staff who are directly or indirectly involved in patients' care, and the CHOP IRB.

9.4 Records Retention

Study documents and data will be retained in accordance with CHOP's retention policy.

9.5 Regulatory and Ethical Considerations

9.5.1 Data and Safety Monitoring Plan

An Investigational New Drug (IND) Exemption has been obtained from the University of Pennsylvania IND/IDE Support Unit within the Penn 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, Natalie Terry, MD, PhD, has been identified to provide additional monitoring for the study. This individual is a board-certified gastroenterologist in pediatric 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 CHOP IRB guidelines, all onsite, unanticipated SAEs will be reported to the Safety Officer promptly (within 24 hours) 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.

During the trial, study medications will be paused in the event of a serious adverse event that qualifies as a CTCAE Grade 3 or Grade 4 and is definitely or probably related to the study

intervention. During the pause of study enrollment, the study team will review the SAE with the CHOP IRB and the Safety Officer to determine whether study procedures should be continued or stopped. If either entity determines that the study should be stopped, all recruitment, enrollment, sample collection and follow-up will be halted. Participants will be transitioned to standard of care under the guidance of their treating physician. His/her data will still be used in the final analyses.

9.5.2 Risk Assessment

9.5.2.1 Group 1 Participation

There are potential risks to participants who agree to participate in this trial; however, the risks are relatively small and have been minimized by study design. Group 1 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. 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.

Risks Associated with Study Medications

The therapeutic regimen described in this protocol is safe. These are medications that are already FDA approved for various indications. The protocol is using standard dosing. Also, many of the medications in the proposed regimen are already utilized in children and adults with CD. In the preliminary data, it was found a similar regimen to be safe in primates. No SAEs related to the drug regimen have been reported for the five subjects enrolled in the IRB-approved protocol being conducted through Penn.

Subjects with a known allergy to any of the study medications will not be able to participate.

Subjects who do not know how they will react to the study medications are at risk for an allergic reaction including anaphylaxis. Anaphylaxis is a severe, potentially life-threatening allergic reaction. It can occur within seconds or minutes of exposure to something someone is allergic to. The flood of chemicals released by the immune system during anaphylaxis can cause blood pressure to drop suddenly and airways can narrow, making it hard to breathe normally. Signs and symptoms of anaphylaxis include a rapid, weak pulse, a skin rash, nausea and vomiting. Anaphylaxis requires immediate medical attention including an injection of epinephrine. If anaphylaxis is not treated right away, it can lead to unconsciousness or even death.

For any antibiotic, there is the possibility of "resistance" developing. Resistance means that specific, infection-causing bacteria that were treatable by the antibiotic are no longer as easily treated by that antibiotic.

For any antibiotic, there is also the possibility that subjects could develop *Clostridium difficile*. However, vancomycin, one of the study medications, is used to treat *C. difficile* and therefore, the chances of developing *C. difficile* while on study medications is extremely unlikely.

Known Adverse Reactions to Study Medications

<table>
<tr><th colspan="2">Ciprofloxacin (Cipro)</th></tr>
<tr><td colspan="2">Reactions found in Clinical Trials in Pediatric Populations</td></tr>
<tr><td colspan="2">Common Reactions (occurs in ≥ 5% of patients)</td></tr>
<tr><td>• Temporary mild to moderate negative musculoskeletal reactions:
  ○ Joint pain
  ○ Joint sprains</td><td>○ Decreased range of joints
○ Degeneration of joints
○ Abnormal walking patterns
○ Body and muscle pain</td></tr>
<tr><td colspan="2">Less Common Reactions (occurs in 1-5% of patients)</td></tr>
<tr><td>• Diarrhea
• Vomiting
• Abdominal pain
• Stuffy nose
• Indigestion
• Nausea
• Fever</td><td>• Asthma
• Rash
• Dizziness
• Nervousness
• Trouble sleeping
• Drowsiness
• Laboratory value changes</td></tr>
<tr><td colspan="2">Reactions found in Clinical Trials in Adult Populations (possible in pediatric populations)</td></tr>
<tr><td colspan="2">Common Reactions (occurs in 1-2.5% of patients)</td></tr>
<tr><td>• Nausea
• Diarrhea
• Abnormal liver function tests</td><td>• Vomiting
• Rash</td></tr>
<tr><td colspan="2">Rare but Potentially Dangerous Reactions (occurs in <1% of patients)</td></tr>
</table>

• *Body related pain* (i.e. headaches, abdominal pain, foot pain, etc.) • *Blood flow* (i.e. abnormal heart beat, high/ low blood pressure, heart attack, minor bleeds in the skin, swollen lymph nodes etc.) • *Mental state* (i.e. restlessness, dizziness, trouble sleeping, drowsiness, irritability, hallucinations, depression, poor appetite, etc.) • *Gastrointestinal system* (i.e. difficulty swallowing, intestinal perforation, GI bleeding, jaundice, hepatitis, etc.) • *Metabolism and nutritional systems* (i.e. increase in pancreas labs, high or low blood sugar)	• *Musculoskeletal system* (i.e. joint, back, neck or chest pain, joint stiffness, achiness, gout flare up) • *Kidneys, urinary or genital organs* (i.e. kidney inflammation, kidney failure, bloody urine, breast pain, vaginal pain, discharge or itching, etc.) • *Breathing* (i.e. shortness of breath, nose bleeds, fluid in the lungs, inflamed larynx, hiccup, coughing up of blood, blood clot in lungs etc.) • *Skin and sensitivity* (i.e. allergic reaction, fever, chills, sweating, itchy skin, sensitivity to the sun, flushing, swelling of the face, neck, lips etc.) • *Senses* (i.e. blurred vision, change in color perception, vision loss, double vision, hearing loss, bad taste etc.)

Vancomycin	
Reactions found in Clinical Trials in Adult Populations (possible in pediatric populations)	
Common Reactions (occurring in ≥ 5% of patients)	
• Nausea • Abdominal pain • Vomiting • Diarrhea • Gassiness • Decreased potassium levels • Fever	• Swelling of feet, ankles, or legs • Fatigue • Urinary tract infection • Back pain • Headache • Kidney failure • Increased kidney laboratory values
Additional possible reactions. The likelihood of having these side effects is unknown.	
• *Toxicity to the ear:* (i.e. hearing loss (associated with IV therapy), vertigo, dizziness, hearing extraneous sounds i.e. ringing, clicking etc.)	• *Blood cells:* (i.e. reversible low white blood cell count) • *Miscellaneous* (i.e. anaphylaxis, chills, rash, life-threatening skin condition, blood vessel destruction by inflammation)

Neomycin	
Reactions reported during treatment with Neomycin	
Common Reactions	
• Nausea • Diarrhea	• Vomiting
Other Adverse Reactions	
• *Brain system:* (i.e. nerve damage, damage to the inner ear (which could lead to temporary or permanent hearing loss and balance), paralysis of the nerve and muscle systems, which may cause death, severe difficulty breathing, numbness, skin tingling, muscle twitching and convulsions) • *Kidney:* (i.e. damage to kidney, kidney failure)	• *Neonatal: (*i.e. harm to an unborn baby when distributed to a pregnant woman, permanent hearing loss at the time of birth) • *Miscellaneous:* (i.e. yeast infection and other similar infections, inability to absorb certain nutrients, leading to increased fat, nitrogen, cholesterol, carotene, glucose, xylose, lactose, sodium, calcium, cyanocobalamin and iron in the stool)

Fluconazole	
Reactions found in Clinical Trials in Pediatric Populations	
Common Reactions (occurs in 1-5% of patients)	
• Vomiting • Abdominal Pain • Nausea	• Diarrhea • Abnormal liver function tests
Reactions found in Clinical Trials in Adult Populations (possible in pediatric populations)	
Common Reactions (occurs in 1-5% of patients)	
• Headache • Nausea • Skin rash • Abdominal pain • Vomiting	• Diarrhea • Dyspepsia • Dizziness • Taste perversion • Abnormal liver function tests
Rare but Potentially Dangerous Reactions	

• *Liver:* (i.e. gallbladder inflammation, yellowing of the skin, liver damage, serious liver infections like hepatitis, sudden liver failure which could lead to death in those with serious underlying medical conditions)	• *Miscellaneous* (i.e. anaphylaxis, rapid swelling of the skin, birth defects when taken during early pregnancy)
Other Post-Marketing Adverse Reactions	
• *Heart* (i.e. irregular heart rhythms, abnormal heart rhythm disorders) • *Blood cells* (i.e. decreased white blood cells and platelets) • *Body* (i.e. abnormal weakness, lack of energy, tiredness, discomfort or uneasiness, fever) • *Brain* (i.e. seizures, dizziness, sleeping problems, sleepiness, tremor, vertigo, nerve damage- characterized by numbness or tingling, tickling, prickling, burning etc. of the skin without a physical cause)	• *Skin* (i.e. rash caused by drug reaction, life-threatening skin conditions, increased sweating, hair loss) • *Metabolism and nutritional system* (i.e. increased blood cholesterol, increased blood triglycerides, low blood potassium) • *Gastrointestinal* (i.e. blockage of bile to the duodenum, dry mouth, indigestion, vomiting, changes in taste) • *Miscellaneous* (i.e. anaphylaxis, rapid swelling of the skin, swelling of the face, severe itching of the skin, liver damage, muscle pain)

MiraLax (PEG 3350)	
Reactions reported during treatment with MiraLax (PEG 3350)	
Common Reactions	
• Nausea • Abdominal Bloating • Cramping	• Flatulence • Diarrhea and excessive stool frequency with high doses • Hives

Reproductive Risks

It is unknown what effect these treatments may have on an unborn child. Subjects of childbearing age will be asked to practice an effective method of birth control while participating in this study. Potential subjects will be made aware that taking oral antibiotics can potentially lower the effectiveness of oral birth control. If a subject becomes pregnant

during this study, as soon as their doctor is made aware, they will be asked to stop taking study medicine and will be withdrawn from the study.

Blood Draws

Blood draws can cause discomfort, bruising, have a small risk of infection, or a blood clot. Standard methods will be used to prevent infection.

Stool Collection

Any stool sample may contain germs that spread disease. Subjects will be asked to carefully wash their hands and use careful handling techniques to avoid spreading infection. Subjects will be provided with directions on how to properly and safely package and ship samples (if applicable).

Rectal Swab Collection

Subjects may experience minor physical discomfort while having the swabs taken.

EKG

Subjects may experience minor physical discomfort or anxiety while having the EKG procedure.

9.5.2.2 Group 2 Participants

The medical, physical and social risks are minimal for subjects of this arm of the study. The primary risk is a breach of privacy and confidentiality, but we will take the measures noted in the main study to ensure privacy and confidentiality.

Stool Collection

Collecting stool may contain germs that spread disease. Subjects will be reminded to carefully wash their hands and use careful handling techniques to avoid spreading infection. Some people may feel uncomfortable or embarrassed when supplying a stool sample. There should be no pain while collecting the stool sample. However, if a subject is constipated, straining to pass stool may be painful. We will discuss this with the subject during the recruitment process and ensure that the individual subject is comfortable with the process.

Breach of Confidentiality

As with any study involving collection of data, there is the possibility of breach of confidentiality of data. Every precaution will be taken to secure participants' personal information to ensure confidentiality.

At the time of participation, each participant will be assigned a study identification number. This number will be used on data collection forms and samples and in the database instead of names and other private information.

9.5.3 Potential Benefits of Trial Participation

9.5.3.1 Potential Benefits to Group 1 Participants

The direct benefit is improvement in disease activity and potential salvaged response to the prior treatment regimen (immunomodulator or biologic medication). In terms of indirect benefits, participation in this study will improve the understanding of the role of the gut microbiota in the inflammatory bowel diseases. 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.

9.5.3.2 Potential Benefits to Group 2 Participants

There are no direct benefits to the individual participants in this study. Primarily, the study has considerable potential benefit for all patients with IBD by better elucidating the effect of bowel lavage on the gut microbiota and the gut inflammatory marker, fecal calprotectin.

9.5.4 Risk-Benefit Assessment

9.5.4.1 Group 1

The potential benefits outweigh any risks of participating in this study, as the treatment may help to salvage response to prior treatment regimens. The risks of participating in this study are small, and these risks are outweighed by the benefits to society to be gained from new knowledge about the gut microbiota and how it may play a role in inflammatory bowel disease management.

9.5.4.2 Group 2

There are no direct benefits to the subjects for their participation in this group of the study. However, the risks of participating are minimal, and these risks are outweighed by the potential benefit of improving diagnosis and treatment of children with IBD.

9.6 Recruitment Strategy

9.6.1 Group 1 Participants

Potential participants will be identified through outpatient clinic schedules, inpatient lists, endoscopy schedules and physician referrals. The study team will reach out to GI specialists at various hospital systems, such as St. Christopher's Hospital for Children and Nemours Alfred I. duPont Hospital for Children, to request referrals and help propagate information about the study to potential subjects. Potential subjects may be provided with the study team's contact information to contact CHOP directly. Non-CHOP physicians may also obtain permission from potential subjects to release limited contract information to the study team so they may contact interested individuals.

Additionally, families may be approached at CHOP sponsored education meetings and events. Appropriate follow up from the study team may occur by phone or email.

Contact information for the research team will be provided and a link to www.clinicaltrials.gov for more information.

An IRB approved flyer may also be distributed to potential participants. This document is attached in section 12.02 (1.0) of the IRB application. Flyers may be mailed, emailed, or handed out in-person.

We also may be contacted through various websites and social media platforms, such as www.clinicaltrials.gov, the Crohn's and Colitis Foundation of America (CCFA) Clinical Trial Registry, and the CHOP Clinical Research Finder public website by individuals outside of CHOP. Web-based advertising avenues, like eNewsletters, 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).

We will conduct a social media campaign via the CCFA Facebook page. The Facebook posts will guide potential participants to contact IBDResearch@email.chop.edu to receive more information about the study. The study's posts will be managed by the CCFA, who have their own team of experts to monitor posts/comments.

There may be additional social media recruitment campaigns through the CHOP recruitment enhancement core or though the University of Pennsylvania. All materials and language posted will obtain IRB approval. 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.

9.6.2 Group 2 Participants

Potential participants will be identified through outpatient clinic schedules, inpatient lists, endoscopy schedules and physician referrals. Potential participants may be approached via phone, email, or at CHOP sponsored education meetings and events. Contact via email will be done with IRB approved language. Appropriate follow up from the study team may occur by phone or email. 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.

9.7 Informed Consent/Assent and HIPAA Authorization

Study personnel will obtain consent and assent for the study. Subjects may make a decision about study participation at that time, or may decide to enroll at a later visit.

The study will be thoroughly explained by study personnel, including the study rationale and goals.

For participants enrolled in Group 1, a physician on the study will explain the risks and benefits of the intervention and study procedures that are greater than minimal risk. A physician or another member of the research team, with consenting authority, will explain the rest of the consent form. Potential participants will be given the opportunity to ask questions to the physician and other study personnel about the study, risks, benefits, and confidentiality. Written consent will be obtained after all the questions and discussions have been completed. If a physician on the research study has not personally completed the consent form, signed and dated it himself/herself, a note will be included in either the

medical record or study record indicating that the consent conversation occurred. For the subjects under 18 years of age, assent will be obtained by explaining briefly the study while the parent/guardian is present. Participation in all areas of the study will be completely voluntary. Should the subjects become intolerant of any aspect of the study, their participation will be discontinued. Subjects who turn 18 during the course of the study will be re-consented.

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.

9.7.1.1 Verbal Consent & Waiver of Documentation of Consent

We are requesting to obtain verbal consent and waiver of documentation of consent for participants enrolled in both Group 1 and Group 2 of the study.

9.7.1.1.1 Group 1

For participants recruited through physician referrals or for potential subjects who are not at CHOP prior to enrollment, they may be contacted and screened via telephone to obtain verbal consent. Once verbal consent is obtained, potential participants or their parent/guardian will be emailed a REDCap Screening Questionnaire. Participants who seem to meet all inclusion criteria (see Section 3.4) will be scheduled for an enrollment visit. The purpose of the verbal screening informed consent is to confirm the participant's eligibility and obtain an IBD history. It is also to allow subjects to collect samples at home prior to their visit. Subjects are not always able to have a bowel movement while at the hospital and therefore, it will be helpful for subjects to collect the stool sample at home prior to their appointment. A stool collection kit will be given to or mailed to all participants and/or their parent/guardian so that a sample can be brought to the enrollment visit.

When consent is obtained by telephone, the person obtaining consent will document the participant's agreement to participate on the verbal consent form. We could not practicably carry out the research without the verbal consent, because it is possible that not all subjects will be seen at the main hospital within the timeframe of study recruitment. At the enrollment visit, the study will be reviewed in its entirety with the participant and the participant's parents/guardian. Written consent and assent will be obtained. No samples will analyzed before obtaining written consent.

9.7.1.1.2 Group 2

As with those in Group 1, potential Group 2 participants may be contacted and screened via telephone to obtain verbal informed consent. The purpose of the verbal informed consent is to allow participants to collect a stool sample at home prior to their clinically-indicated PEG lavage. We could not practicably carry out the research without the verbal consent and waiver of documentation of consent as subjects do not typically come to the hospital to schedule a procedure and undergo a PEG lavage for clinical purposes. Instructions on when and how to undergo the lavage is given over the phone.

Subjects consented by telephone with the verbal consent with have the study procedures reviewed in their entirety before obtaining verbal consent. It will be made

clear to the potential subjects that they can refuse participation and it will not impact their care at CHOP. The subject will be given the opportunity to receive a copy of the consent. For subjects being consented via the telephone the following sections on waiver of assent, and waiver of HIPAA authorization apply.

9.7.1.2 Waiver of Assent

A full waiver of assent is requested for the verbal consent forms (Verbal Screening Informed Consent Form; to be used with Group 1 participants only and the Verbal Informed Consent Form; to be used with Group 2 participants only) which can be done over the telephone with parents/guardian and children may not be available.

9.7.1.2.1 Group 1

At the enrollment visit, the study will be reviewed in its entirety with the participant and the participant's parents/guardian. Written assent will be obtained at this in-person visit and documented on the main consent form

All Group 1 participants and/or their parent/guardian must provide written consent before undergoing any other study procedures. The study procedures will be explained to each participant and their parent/guardian 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 visit.

9.7.1.3 Waiver of HIPPAA Authorization

A partial waiver of HIPAA authorization to obtain verbal authorization is requested for both Group 1 and Group 2 participants. The waiver would allow Group 1 participants to collect the appropriate samples prior to their enrollment visit and enrolled Group 2 participants to collect samples prior to being seen at CHOP or for subjects who will not require a visit to CHOP.

9.8 Payment to Subjects/Families

All participants will be provided compensation for their enrollment in this study. Participants will be compensated $10.00 per stool sample submitted on a pre-paid debit card. If all sample collections are completed an addition $70 will be provided. Group 1 participants can earn a maximum of $120 for their participation in the study. Group 2 participants can earn a maximum of $100 for their participation in the study. If a family is asked to repeat sample collection due to improper collection initially, the family will not be compensated for repeat samples. The bank issuing the debit card will have access to identifiable information. The bank will not have access to any medical information.

9.8.1 Gifts

Group 1 participants will be given a pitcher to prepare the Miralax (PEG 3350) solution for the day 2 bowel lavage.

10 PUBLICATION

Data from this study, including preliminary data analyses, will be presented at national meetings and submitted for publication. Confidentiality will be maintained during publication.

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