A Multicenter Randomized, Double-Blinded, Placebo-Controlled Study of Posaconazole in Genetically-Defined Patients With Active Crohn's Disease

NCT04966585

February 23, 2023

A MULTICENTER RANDOMIZED, DOUBLE-BLINDED, PLACEBO-CONTROLLED STUDY OF POSACONAZOLE IN GENETICALLY-DEFINED PATIENTS WITH ACTIVE CROHN'S DISEASE

An Investigator-Initiated Study

Funding Sponsor: *NIDDK*

Study Product: Posaconazole (Noxifil®)

Protocol Number: 7.0

Current version: ~~February 4, 2021~~

~~August 16, 2021~~

~~October 15, 2021~~

~~January 21, 2022~~

~~October 31, 2022~~

February 23, 2023

Summary of Changes – 23-Feb-2023

- Pages 2 & 20 – Updated Inclusion #1 male and female patients age ≥ 18, there will no longer be a cutoff age.

Table of Contents

List of Abbreviations

AE	Adverse Event
CD	Crohn's Disease
CXR	Chest radiograph
EKG	Electrocardiogram
EOS	End of Study
EOT	End of Treatment
FDA	Food and Drug Administration
GWAS	Genome-wide association study
hsCRP	High-sensitivity C-reactive protein
IBDQ	Inflammatory bowel disease questionnaire
IL	Interleukin
IRB	Institutional Review Board
ITT	Intention-to-Treat
PK	Pharmacokinetic
UC	Ulcerative Colitis
SES-CD	Simple Endoscopic Score for Crohn's Disease
SF-36	Short Form 36

Protocol Synopsis

Title	Posaconazole foR gEnetiCally-defIned crohn'S dIsease Of the termiNal ileum (PRECISION): A two-center, placebo-controlled prospective pilot study
Short Title	Pilot Study of posaconazole in Crohn's disease
Protocol Number	*TBD*
Sponsor	Investigator-initiated
Indication	Posaconazole as treatment for active Crohn's disease in people with active Crohn's disease (CD) despite conventional therapy who are at risk for fungal infection based on genetically determined CARD9 S12N allele status
Background	The etiology of Crohn's disease is thought to involve abnormal gut immune response to commensal flora in genetically-susceptible individuals. Several studies have implicated commensal fungi, including *Malassezia* spp with the development of CD. Innate inflammatory responses to fungi are mediated in large part through a signaling pathway involving a protein called CARD9. Genome-wide association studies (GWAS) have linked a polymorphism in *CARD9* (S12N) to the risk of CD, and we have found that the presence of *Malassezia* was positively linked to the presence of the *CARD9* risk allele. We further found that the disease-associated risk allele of *CARD9* specifically enhances human inflammatory responses to *Malassezia* and that *Malassezia* exacerbates colitis via *CARD9* in mouse models of disease. This trial is designed to evaluate the effects of oral antifungal treatment with posaconazole on disease activity and the burden of *Malassezia* spp. in CD patients with the *CARD9* S12N allele. Further, this project will investigate the hypothesis that the microbial changes induced by antifungal treatment are associated with dampened downstream immune responses in those with a genetic predisposition to developing strong immune responses to *Malassezia.*
Design	Randomized, double-blinded, placebo-controlled trial
Study Duration	Four-week screening period followed by twelve-week double-blind induction period, followed by either 1) a 6-month follow-up period or 2) (for placebo non-responders only) a twelve-week open-label period followed by a twelve-week follow-up.
Study Center(s)	Two-centers: Cedars-Sinai Medical Center, Mayo Clinic (Rochester)

Objectives	*Primary Objective* • To assess the effects of posaconazole on Crohn's disease activity in patients with active Crohn's disease *Secondary Objectives* • To assess the preliminary safety and tolerability of posaconazole for the induction of clinical remission and endoscopic response in patients with active Crohn's disease with genetically-determined CARD9 S12N risk allele • To assess the preliminary influence on quality-of-life of posaconazole in patients with active Crohn's disease with genetically-determined CARD9 S12N risk allele • To assess the pharmacokinetics (PK) of posaconazole in patients with active Crohn's disease with genetically-determined CARD9 S12N risk allele • To assess changes in the intestinal mycobiome after treatment with posaconazole in patients with active Crohn's disease with genetically-determined CARD9 S12N risk allele • To determine changes in gene expression (transcriptomics) in colonic mucosa after posaconazole in patients with active Crohn's disease with genetically-determined CARD9 S12N risk allele
Number of Subjects	24
Diagnosis and Inclusion Criteria	Subjects (n=24) will be individuals with active Crohn's disease (SES-CD ≥ 6 AND Crohn's disease activity index score >220) despite use of conventional therapy. In this 40-week trial, subjects will be administered posaconazole (Noxifil®, Merck) 300mg twice daily for 1 day followed by 300mg daily for 12 weeks, or matching placebo. At 12 weeks, subjects will undergo assessment for response, and treatment assignments will be unblinded. Based on active disease criteria at the end of induction and treatment assignment, subjects will either be observed off posaconazole over the next 24 weeks, or (if they are deemed to be a 'non-responder' AND were assigned to placebo) will have the option to enroll into a twelve-week open label period, followed by a twelve week follow up period. In addition to the treatment intervention, subjects also will provide samples for parallel translational studies including blood for genetic analysis, stool samples for biomarker and microbial composition analysis, and biopsies for mucosal gene expression analysis. Inclusion Criteria 1. Male or female patients ≥ 18 years of age. 2. A diagnosis of CD with minimum disease duration of 6 months with involvement of the ileum and/or colon documented on colonoscopy 3. Have an endoscopically-confirmed active Crohn's disease with active disease defined by SES-CD ≥ 6 (≥4 if ileal only), AND active symptoms of Crohn's disease (CDAI score >220) at Baseline Visit.

Inclusion Criteria (continued) and Exclusion Criteria	Inclusion Criteria (continued) 4. Subjects receiving oral aminosalicylates (at a stable dose for 2 weeks prior to baseline), immunomodulators (at a stable dose for 4 weeks prior to baseline), anti-TNF, anti IL12/23, or anti-integrin therapy (at stable maintenance doses for ≥ 8 weeks) may continue their use during the study. 5. Subjects receiving oral corticosteroids may continue their use during the study provided the dose (prednisone up to 20 mg/day, budesonide up to 9 mg/day) has been stable for two weeks prior to screening. 6. Lab findings of hypokalemia, hypomagnesemia, or hypocalcemia are eligible, provided electrolyte abnormalities are corrected and stable prior to study enrollment. 7. Have had age-appropriate and disease-duration-appropriate colon cancer screening without unresected dysplasia. 8. Women of childbearing age, excluding those with prior bilateral tubal ligation or at least one-year post-menopause, must not be pregnant, lactating, or planning to become pregnant. They must agree to use effective contraception throughout the study period. 9. Subjects must be able to provide informed consent and understand, agree with, and be able to adhere to daily diary entries, all scheduled visits, tests, procedures, and protocol in English. Exclusion Criteria The presence of any of the following will **exclude** a subject from enrollment: 1. Known hypersensitivity or allergy to posaconazole or other azole antifungal agents 2. Concomitant medications primarily metabolized by CYP3A4 including (but not limited to): a. HMG-CoA inhibitors primarily metabolized by CYP3A4 (increases risk of rhabdomyolysis) b. Sirolimus c. Ergot alkaloids d. Vincristine e. upadacitinib 3. Proarrhythmic conditions, including baseline EKG demonstrating QTc > 460msec 4. Moderate or severe renal impairment (Cr Clearance <50) 5. Elevated transaminases or bilirubin > 2.5x ULN. 6. Current diagnosis of ulcerative colitis, indeterminate colitis, ischemic colitis, infectious colitis, or microscopic colitis. 7. Fulminant colitis, toxic megacolon, peritonitis, ileostomy or colostomy. 8. Stool sample positive for pathogens including ova and parasites, *Salmonella*, *Shigella*, and *C. difficile* at screening. 9. History of any clinically significant neurological, renal, hepatic, gastrointestinal, pulmonary, metabolic, cardiovascular, psychiatric, endocrine, hematological disorder or disease or any other medical condition that, in the Investigators opinion, would prevent the subject from participation in the study. 10. Treatment with antibiotics, antifungal agents, probiotics, or prebiotics within two weeks of screening. 11. Alcohol or drug abuse (in the opinion of the Investigator) that would interfere with compliance. Any other investigational therapy or treatment within four weeks of screening.

Study Product, Dose, Route, Regimen	Posaconazole (Noxifil®) delayed-release over-encapsulated tablets: Loading dose: 300mg (three 100mg delayed-release over-encapsulated tablets) twice a day on the first day, then Maintenance dose: 300mg (three 100mg delayed-release over-encapsulated tablets) once a day, starting on the second day. Duration of therapy will be 12 weeks.
Duration of administration	Twelve weeks double-blind, with possible twelve-week open label for placebo-treated non-responders at the end of 12 weeks
Primary Endpoint	• The primary endpoint is a 50% decrease in endoscopic disease activity (determined by Simple Endoscopic Score for Crohn's Disease (SES-CD), centrally read) at Week 12
Key Secondary Endpoints	• The proportion of subjects in clinical remission at Week 12, defined by the Crohn's Disease Activity Index (<150) • The proportion of subjects in endoscopic remission at week 12, defined by SES-CD ≤ 3 • The safety of posaconazole, determined by assessment of AEs and concomitant medications throughout the duration of the study.
Safety Endpoints	• Incidence and severity of adverse events • Change from baseline of laboratory values (chemistry, hematology, electrolytes, liver enzymes, renal function) • Incidence of electrocardiogram (EKG) abnormalities including QTc prolongation
Quality-of-life Endpoints	• Inflammatory bowel disease questionnaire (IBDQ) • Short Form SF-36 (SF-36)
Pharmacokinetic Endpoints	• Serum posaconazole concentrations at baseline, and every 2 weeks during the 12-week treatment period • Relationship between posaconazole serum concentration and clinical and antifungal effects
Intestinal Microbiota Endpoints	• Malassezia eradication, determined by a 95% decrease in *Malassezia* spp. in colonic mucosal washings at week 12, detected by qPCR.
Transcriptomics Endpoints	• Changes in stool and mucosal washing metabolites • Changes in microbiome / metabolome reflected in gene expression patterns in mucosal biopsies

Statistical Methodology	The primary analysis population will be the intention-to-treat population. The primary efficacy endpoint and key secondary endpoints will also be analyzed with the per-protocol population. The primary and key secondary endpoints will be evaluated using descriptive statistics, Exact Fisher's and t-test to compare treated vs placebo-controlled subjects. . In the case of missing clinical data, the missing data points will be treated by non-responder imputation. The primary endpoint is endoscopic response, defined as a 50% reduction SES-CD scores at week 12. Each patient will be scored on a binary response (improved/not improved). We will assume the placebo response rate of approximately 10% [2-4]. We estimate that a study size of 24 patients is necessary, assuming a drop-off rate of ~10% before the second colonoscopy, to yield final analysis of at least 11 subjects per arm. With a final sample size of 22 subjects, we will have76% power to detect a difference of 0.50 in the rate of response for treatment with posaconazole therapy vs placebo from the assumed underlying placebo response rate at the 0.05 significance level in a Fisher's Exact Test. This assumes the treatment response rate will be 0.70h or greater. Secondary endpoints will include clinical remission (defined as Crohn's disease activity index < 150), analysis of data regarding use of corticosteroids, mucosal healing, mucosal improvement, normalization of inflammatory biomarkers, and improvement in health-related quality of life. Repeated measures analysis will be used to examine changes over time from baseline. Categorical data will be analyzed with repeated measures logistic regression while continuous measures will used mixed modeling regression. Residuals will be inspected to confirm data meets assumptions necessary for parametric testing. Data will be considered significant at the two-sided p-value of <0.05. All analysis will be performed by the study biostatistician (C Bresee, MS) using SAS v9.4 software.

Table 1: Overview of Study Schedule

Study Assessments	Screening	Baseline	Treatment						Follow-up					
	Week -4 to day -1	Week 0/Day 0	Week 2±3 days	Week 4±3 days	Week 6±3 days	Week 8 ±3 days	Week 10 +/- 3 days	Week 12 (or early termination[1]) ±3 days	Week 16±3 days	Week 20±3 days	Week 24 ±3 days	Week 28 ±3 days	Week 32 ±3 days	Week 36 ±3 days
Informed Consent	X													
Medical History[2]	X													
Eligibility Assessment[3]	X	X												
Height + weight	X													
Vital Signs	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Concomitant Medications	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Complete Physical Examination	X	X						X						X
Targeted Physical Examination[4]			X	X	X	X	X		X	X	X	X	X	
Daily diary instructions	X	X												
Laboratory Studies														
Blood draw for genetics[13]	X													
Serum pregnancy test[5]	X													
Urine pregnancy test[5]		X	X	X	X	X	X	X	X[12]	X[12]	X[12]	X[12]	X[12]	
Pharmacokinetics laboratory studies			X	X	X	X	X	X						
High sensitivity CRP		X	X	X	X	X	X	X	X	X	X	X	X	X
Safety laboratory tests[7]	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Stool studies for pathogens[8]	X													
Stool calprotectin		X	X	X	X	X	X	X	X	X	X			X

Stool collection for microbiome analysis	X	X	X	X	X	X	X	X	X	X	X			X
Whole blood for Transcriptomics	X							X						X
Imaging and Procedures														
EKG[9]	X		X					X	X[12]	X[12]	X[12]	X[12]	X[12]	
CXR[9]	X													
Colonoscopy[10]	X							X						X
Mucosal Washing	X							X						X
Mucosal biopsies for RNA Later & Formalin	X							X						X
Study therapy management														
Study therapy dispensed		X	X	X	X	X	X	X[11]	X[11]	X[11]				
Study therapy accountability			X	X	X	X	X	X	X	X	X			
Adverse events assessment		X	X	X	X	X	X	X	X	X	X	X	X	X
Crohn's disease assessments														
Bowel movement diary	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Crohn's Disease Activity Index		X	X	X	X	X	X	X	X	X	X	X	X	X
PRO2		X	X	X	X	X	X	X	X	X	X	X	X	X
SES-CD score	X							X						X
Quality-of-Life assessments														
IBDQ		X	X	X	X	X	X	X	X	X	X	X	X	X
SF-36		X	X	X	X	X	X	X	X	X	X	X	X	

[1]Subjects who discontinue early will undergo all tests and procedures according to the Week 12 visit at the time of discontinuation or as soon as possible thereafter
[2]Medical history include date of symptom onset, date of diagnosis, and medication/surgical history. Medical history is detailed in Section 6.

[3]The first eligibility assessment will be performed after all other required study assessments for the screening visit except colonoscopy. The second eligibility assessment will be performed after review of concomitant medications, urine pregnancy test, and colonoscopy.
[4]Targeted physical examination focuses on symptoms, as well as on eyes, throat, lymph nodes, cardiopulmonary systems, abdomen, musculoskeletal, extremities, and skin.
[5]Only for females with childbearing potential excluding those with bilateral tubal ligation or at least one-year post-menopause.
[7]Safety laboratory tests include serum chemistries, complete blood count with automatic differential, electrolyte panel (potassium, magnesium, calcium, sodium, chloride, phosphate), lipid panel
[8]Stool studies include stool ova and parasitology, culture, and *C. difficile* toxin assay Drawn only if not performed in the last 4 weeks
[9]These tests will be read by non-study physicians. Chest Xray posterior/anterior and lateral if not performed in the last 12 months
[10]Baseline colonoscopy will be performed between Week -4, Day 1 and Week 0, Day 1, after eligibility assessment. Bowel prep will be dispensed or prescribed at Week -4.
[11]Dispensing for patients participating in Open-label
[12]Only required for participants receiving Open-label drug
[13] gene testing will only be performed on patients whose CARD9 status is unknown.

1.1 Background

Inflammatory bowel disease (IBD) is the result of a dysregulated inflammatory immune response in the mucosa of the gastrointestinal tract. Innate and adaptive immune cells drive disease by producing inflammatory mediators that sustain local inflammatory responses and promote expression of adhesion molecules that direct trafficking of further immune cells into the gut. The disease is associated with alterations in the permeability of the intestinal epithelia and in the intestinal microbiota (intestinal "dysbiosis"). IBD is usually classified as Crohn's Disease (CD) or ulcerative colitis (UC) based on phenotypic features of disease location, "patchiness", and depth of damage to the gastrointestinal (GI) tract. Patients can experience diarrhea, fever, fatigue, pain and cramping, blood in the stool, loss of appetite and weight loss. They may experience periods of active illness followed by periods of clinical remission, although cumulative bowel damage occurs leading to complications. These can include bowel obstruction and fistula formation, often requiring surgical resection of parts of the small or large bowel.

IBD affects an estimated 1.6 million Americans (1 in 200 people in the U.S.) and more than 3.5 million people worldwide. Furthermore, the incidence of IBD is increasing globally[5]. IBD can affect anyone, although it often runs in families suggesting a genetic predisposition. Caucasians are affected at a higher prevalence than other ethnic groups, especially among Jews of European descent (Ashkenazi Jews). However, the incidence of IBD among non-Caucasians including African Americans and Hispanics in the United States is increasing[6]. IBD is also an expensive condition due to the costs of medication, hospitalization, and surgery; the total annual financial burden of IBD in the United States has been figured to be between $14.6 and $31.6 billion[7]. There is no known "cure" for IBD.

Precision care
Rather than being a single disease, or two diseases (CD or UC), IBD is increasingly understood as a multifaceted disease that is initiated and exacerbated by influences such as genetic susceptibility, environmental factors such as diet and smoking, differing immune responses, and alterations in the intestinal microbiota. A major goal in IBD research and care is to improve outcomes by targeting disease treatment to an individual patient's specific underlying disease mechanism(s). The heterogeneity of IBD has prompted searches for a marker or combination of markers to distinguish IBD from non-IBD, differentiate IBD subtypes (e.g. UC versus CD), anticipate disease outcomes, and predict response to therapies.

Genetic diversity
Genetic diversity is increasingly understood to play an important role in susceptibility to developing IBD and in severity of the disease. Large Genome-Wide Association Studies (GWAS) have implicated over 200 genetic polymorphisms in contributing to IBD. These associations reveal specific biological pathways to be involved in disease such as innate immunity (e.g. *NOD2*, *CARD9*), mucosal immunity (e.g. *TNFSF15*, *IL23R*), autophagy (e.g. *ATG16L1*, *IRGM*), and epithelial barrier function (e.g. *COL7A1*, *GUCY2C*). However, translating genetic observations into actionable decision making in clinical care has proven challenging. There are currently no genetic tests in use for informing treatment selection based on disease mechanism, although genetics can inform the safety of thiopurines based on thiopurine methyltransferase (TPMT) enzymatic activity. The search for biomarker identification to stratify patients by likelihood of response to therapy is an active and exciting area of research and development. This project focuses on understanding the role of *CARD9* genotype to target potentially novel therapy.

Serological markers

Serological markers have been used to categorize disease. Perinuclear anti-neutrophil cytoplasmic antibodies (pANCA) have been linked predominantly to UC, and anti-*Saccharomyces cerevisiae* antibodies (ASCA) have been associated with CD[8, 9]. These and other biomarkers can be useful for identifying phenotypic differences and might one day be helpful in making therapeutic choices. For example, clinical response to infliximab (an anti-tumor necrosis factor-α biologic therapy for both CD and UC) may have reduced efficacy in $pANCA^+$ or $pANCA^+/ASCA^-$ patients[10, 11]. Thus, specific antibodies may help to inform therapeutic choices and may provide insights into underlying mechanisms of disease subtypes.

The Microbiome

Intestinal microbes are essential for health of the gut and development of the immune system. Many studies have reported changes in bacterial communities in the gut associated with IBD. Specifically, decreases in relative abundance of some genera of Bacteroidetes and Firmicutes have been reported as well as enrichment of Enterobacteriaceae[12, 13]. Observations of disease-associated differences in intestinal microbial populations in IBD patients have prompted wide-spread interest in the idea that manipulating the gut microbiome may be beneficial. Still, it is not yet clear whether or when microbiota changes cause or are simply a result of disease. Approaches to manipulating the microbiome include fecal microbiota transplantation (FMT) and use of medications (i.e. antibiotics), probiotics, and prebiotics[13].

Reports from small randomized, controlled clinical trials employing FMT to treat IBD patients are mixed[14-17]. In patients with ulcerative colitis one trial showed no benefit, while three have suggested varying degrees of efficacy. However, the studies used different methods of FMT administration (nasoduodenal infusion, enema, colonoscopy), dose frequency (daily or weekly), and formulation of the FMT product (fresh or frozen, single donor or pooled donors). FMT thus remains an experimental treatment requiring further study. Data are similarly mixed for the use of antibiotics to treat IBD patients. While some metanalyses suggest that antibiotics might be helpful in some patients[18], the data are inconsistent and it is not clear whether the risks of antibiotic use outweigh the putative (mostly short-term) benefits. Uses of pro- and pre-biotics to influence the makeup of the gut microbiome for benefit are popular among patients, but there are insufficient data supporting their efficacy for Crohn's disease. In UC, several studies suggest that bacterial probiotics might provide modest benefit in preventing disease recurrence, although given the variety of probiotics and wide variations in patient profiles, the data are difficult to interpret[12].

Ultimately however, any clinical attempt to manipulate the microbiome for therapeutic benefit will be hindered by our inability to identify the specific patients who would benefit from therapies directly targeting the microbiome. While most studies have focused on evaluating the types of bacteria associated with disease, we have recently presented data suggesting a role for intestinal fungi and host immunity to fungi in intestinal inflammation in mice and humans[19, 20]. As discussed below, we have found that a specific yeast, *Malassezia*, is substantially more present in the intestinal mucosa of patients with Crohn's disease and that this yeast exacerbates disease in mouse models of colitis. Innate immune responses to fungi are mediated in large part by the C-type lectin family of receptors that includes Dectin-1, Dectin-2 and Mincle. These receptors, expressed by nearly all myeloid phagocytes, trigger inflammatory responses upon detecting fungi through a signaling pathway that uses a protein called CARD9 to activate NF-κB. GWAS studies have linked a common polymorphism in *CARD9* to the risk of CD. This non-synonymous polymorphism results in a change in serine 12 to asparagine ($CARD9^{S12N}$)[21-23]. As discussed below, we have observed that this polymorphism causes human phagocytes to respond more aggressively to *Malassezia* and that patients with this polymorphism have

more *Malassezia* in their gut mucosa. In this project, we will work toward the hypothesis that *CARD9* genetics (and possibly fungal-specific serotyping) identify a subset of Crohn's disease patients who would benefit from antifungal therapy targeting reduction of *Malassezia* in the colonic mucosa.

Preliminary Data

Based on the above-mentioned association between anti-*Saccharomyces cerevisiae* antibodies (ASCA) and increasing interest in the role of the microbiome in IBD, we analyzed over 160 mucosal washing samples collected during colonoscopy of patients with Crohn's disease and healthy controls at Cedars-Sinai Medical Center (CSMC) by high throughput sequencing of fungal rDNA "internal transcribed spacer 1" (ITS1) genes. We observed a strong association of *Malassezia* spp. (*M. restricta* & *M. globosa*) with CD.

Malassezia spp. are members of the Basidiomycota phyla of fungi and, while all other members of the Ustilaginomycotina subdivision are plant pathogens, *Malassezia* are commensal skin microbes found on nearly all warm-blooded animals. *Malassezia* have among the smallest of eukaryotic genomes, having only around 4000 genes. They grow mainly as yeasts, although some species can develop hyphae. An important feature of *Malassezia* genomes is the loss of key enzymes required for lipid metabolism, including fatty acid synthase (FAS), Δ desaturase, and Δ enoyl CoA isomerase (26). Therefore, they cannot produce fatty acids themselves and need lipids from the environment for growth. That *Malassezia* spp. might be important in intestinal inflammation is unexpected.

Innate immune responses to fungi are mediated in large part by the C-type lectin family of receptors that includes Dectin-1, Dectin-2 and Mincle [24, 25]. These receptors trigger inflammatory response upon detecting fungi through a signaling pathway that uses a protein called CARD9 to activate NF-κB. GWAS studies have linked a nonsynonymous single nucleotide polymorphism (SNP) in exon 2 of *CARD9* o the risk of CD[21]. This SNP leads to production of a CARD9^{S12N} variant of the protein. The allele frequency of the "minor" N12 coding variant is around 40% and present broadly in all ethnic groups examined. It is slightly less frequent in Africans and slightly more frequent in native South American populations. In Europeans, a rare second polymorphism at the end of exon 11 linked to the CARD9^{N12} variant results in production of a splice variant lacking exon 11 and production of a protein lacking function[26]. While the N12 variant confers increased risk of developing Crohn's Disease, the truncation variant is protective[22, 23]. The *CARD9*^{S12N} polymorphism has also been linked to the risk of developing ulcerative colitis[27], primary sclerosing cholangitis[28], ankylosing spondylitis[29], and immunoglobulin A (IgA) nephropathy[30] – each of these has been associated with inflammatory bowel disease as 'extraintestinal manifestations' in select patients. The high prevalence of the gene variant suggests that it may be protective against certain diseases, most likely specific fungal pathogens, but this has not yet been proven.

We investigated whether this polymorphism was linked to the presence of specific fungi. Among patients with CD, the presence of *Malassezia* was positively linked to the presence of the *CARD9* risk allele. To investigate whether the *CARD9* risk allele altered how these patients respond to fungi, we collected blood from healthy volunteers. homozygous for the *CARD9* risk allele or protective allele (via the "Material and Information Resources for Inflammatory and Digestive Diseases" (MIRIAD) biobank at CSMC, the largest repository of IBD specimens in the world). We stimulated monocyte-derived dendritic cells with *Malassezia* and other yeasts and found that the risk allele made the cells respond more vigorously.

To test whether *Malassezia* could influence intestinal inflammation, we used DSS and T cell transfer models of colitis in mice and found that *Malassezia* potently exacerbated disease ENREF 17[20]. To evaluate whether oral antifungals can directly affect Malassezia levels in the gut, we treated mice with oral fluconazole and measured depletion of Malassezia from the stool.

Together the data suggest that association of *Malassezia* with the intestinal mucosa may exacerbate disease in a subset of patients who can be identified by their genetics and/or serological markers.

This trial is designed to test the hypothesis that oral antifungal treatment with posaconazole can reduce the burden of *Malassezia* spp. in CD patients with the CARD9S12N allele. Further, this project will investigate the hypothesis that the microbial changes induced by antifungal treatment are associated with dampened downstream immune responses in those with a genetic predisposition to developing strong immune responses to *Malassezia*.

2 Introduction

This document is a protocol for a human research study. This study is to be conducted according to US and international standards of Good Clinical Practice (FDA Title 21 part 312 and International Conference on Harmonization guidelines), applicable government regulations and Institutional research policies and procedures.

2.1 Indication

Posaconazole as treatment for active Crohn's disease despite treatment with conventional therapy among people with the CARD 9 risk allele.

2.2 Investigational Agent

2.2.1 Description

Posaconazole (Noxafil®, MERCK) is a broad-spectrum, second generation, triazole antifungal compound. Posaconazole inhibits 14-alpha demethylase, a common fungal enzyme necessary for conversion of lanosterol to ergosterol, a component of fungal cell walls. Inhibition of ergosterol synthesis changes the fungal cell membrane composition and integrity, alters membrane permeability and leads to fungal cell lysis. Posaconazole is approved for treatment of mucocutaneous candidiasis, invasive aspergillosis, histoplasmosis, coccidiomycoses, fusariosis, cryptococcosis, periorbital cellulitis due to Rhizopus, and for prophylaxis against these infections in susceptible patients. Posaconazole is available in both parenteral and oral formulations, including delayed-release tablets and oral suspension.

2.2.2 Pharmacokinetics of orally administered posaconazole

Mechanism of Action:
Posaconazole blocks the synthesis of ergosterol, a key component of the fungal cell membrane, through the inhibition of cytochrome P-450 dependent enzyme lanosterol 14α-demethylase responsible for the conversion of lanosterol to ergosterol in the fungal cell membrane. This results in an accumulation of methylated sterol precursors and a depletion of ergosterol within

the cell membrane thus weakening the structure and function of the fungal cell membrane. This may be responsible for the antifungal activity of posaconazole. The azole family of antifungal medications have been shown to inhibit the expression of TNF-alpha-induced cell adhesion molecules and angiogenesis, and have to improve colitis in rat models[31, 32 33], and a case series of 6 patients with inflammatory bowel disease describes unexpected clinical improvement in Crohn's disease after long-term (6 to 9 months) itraconazole treatment for fungal infections[34].

General Pharmacokinetic Characteristics (See Noxafil®, package insert)

Noxafil® delayed-release tablets exhibit dose proportional pharmacokinetics after single and multiple dosing up to 300 mg. The mean pharmacokinetic parameters of posaconazole at steady state following administration of Noxafil delayed-release tablets 300 mg twice daily (BID) on Day 1, then 300 mg once daily (QD) thereafter in healthy volunteers and in neutropenic patients who are receiving cytotoxic chemotherapy for AML or MDS or HSCT recipients with GVHD are shown in **Table:**

.

	N	**AUC0-24 hr (ng·hr/mL)**	**Cav† (ng/mL)**	**Cmax (ng/mL)**	**Cmin (ng/mL)**	**Tmax ‡ (hr)**	**t1/2 (hr)**	**CL/F (L/hr)**
Healthy Volunteers	12	51618 (25)	2151 (25)	2764 (21)	1785 (29)	4 (3-6)	31 (40)	7.5 (26)
Patients	50	37900 (42)	1580 (42)	2090 (38)	1310 (50)	4 (1.3-8.3)		9.39 (45)

CV = coefficient of variation expressed as a percentage (%CV); AUC_{0-T} = Area under the plasma concentration-time curve from time zero to 24 hr; C_{max} = maximum observed concentration; C_{min} = minimum observed plasma concentration; T_{max} = time of maximum observed concentration; $t_{½}$ = terminal phase half-life; CL /F = Apparent total body clearance
*300 mg BID on Day 1, then 300 mg QD thereafter
† Cav = time-averaged concentrations (i.e., $AUC_{0-24\ hr}$/24hr)
‡ Median (minimum-maximum)

Absorption

When given orally in healthy volunteers, posaconazole delayed-release tablets are absorbed with a median Tmax of 4 to 5 hours. Steady-state plasma concentrations are attained by Day 6 at the 300 mg dose (QD after BID loading dose at Day 1). The absolute bioavailability of the oral delayed-release tablet is approximately 54% under fasted conditions. The Cmax and AUC of posaconazole following administration of posaconazole delayed-release tablets is increased 16% and 51%, respectively, when given with a high fat meal compared to a fasted state (see Table 1). In order to enhance the oral absorption of posaconazole and optimize plasma concentrations, posaconazole delayed-release tablets should be administered with food. Concomitant administration of posaconazole delayed-release tablets with drugs affecting gastric pH (Mylanta®, ranitidine (Zantac®), esomeprazole (Nexium®)) or gastric motility (metoclopramide (Reglan®)) did not demonstrate any significant effects on posaconazole pharmacokinetic exposure (posaconazole, package insert).

Table: Statistical Comparison of Plasma Pharmacokinetics of Posaconazole Following Single Oral Dose Administration of 300 mg Posaconazole Delayed-Release Tablet to Healthy Subjects under Fasting and Fed Conditions

	Fasting Conditions		Fed Conditions (High Fat Meal)*		Fed/Fasting
Pharmacokinetic Parameter	N	Mean (%CV)	N	Mean (%CV)	GMR (90% CI)
Cmax (ng/mL)	14	935 (34)	16	1060 (25)	1.16 (0.96, 1.41)
AUC0-72hr (hr·ng/mL)	14	26200 (28)	16	38400 (18)	1.51 (1.33, 1.72)
Tmax† (hr)	14	5.00 (3.00, 8.00)	16	6.00 (5.00,24.00)	N/A

GMR=Geometric least-squares mean ratio; CI=Confidence interval
* 48.5 g fat
† Median (Min, Max) reported for T_{max}

Distribution

The mean volume of distribution of posaconazole after intravenous solution administration was 261 L and ranged from 226-295 L between studies and dose levels. Posaconazole is highly bound to human plasma proteins (>98%), predominantly to albumin.

Metabolism

Posaconazole primarily circulates as the parent compound in plasma. Of the circulating metabolites, the majority are glucuronide conjugates formed via UDP glucuronidation (phase 2 enzymes). Posaconazole does not have any major circulating oxidative (CYP450 mediated) metabolites. The excreted metabolites in urine and feces account for ~17% of the administered radiolabeled dose. Posaconazole is primarily metabolized via UDP glucuronidation (phase 2 enzymes) and is a substrate for p-glycoprotein (P-gp) efflux. Therefore, inhibitors or inducers of these clearance pathways may affect posaconazole plasma concentrations.

In vitro studies with human hepatic microsomes and clinical studies indicate that posaconazole is an inhibitor primarily of CYP3A4. A clinical study in healthy volunteers also indicates that posaconazole is a strong CYP3A4 inhibitor as evidenced by a >5-fold increase in midazolam AUC. Therefore, plasma concentrations of drugs predominantly metabolized by CYP3A4 may be increased by posaconazole. For this reason, the following drugs studied clinically, for which plasma concentrations were affected by posaconazole, will be prohibited during the current proposed trial (cyclosporine, tacrolimus, sirolimus, simvastatin, midazolam, rifabutin, phenytoin, ritonavir, atazanavir *[see Exclusion Criteria*].

Elimination

Following administration of Noxafil oral suspension, posaconazole is predominantly eliminated in the feces (71% of the radiolabeled dose up to 120 hours) with the major component eliminated as parent drug (66% of the radiolabeled dose). Renal clearance is a minor elimination pathway, with 13% of the radiolabeled dose excreted in urine up to 120 hours (<0.2% of the radiolabeled dose is parent drug). Posaconazole delayed-release tablet is eliminated with a mean half-life (t½) ranging between 26 to 31 hours.

2.2.3 Use In Specific Populations

Pregnancy and Lactation
Based on findings from animal data, Noxafil® may cause fetal harm when administered to pregnant women. Available data for use of Noxafil in pregnant women are insufficient to establish a drug associated risk of major birth defects, miscarriage, or adverse maternal or fetal outcomes. Pregnant women will be excluded from the current study.

There are no data on the presence of posaconazole in human milk, the effects on the breastfed infant, or the effects on milk production. Posaconazole is excreted in the milk of lactating rats. Lactating women will be excluded from the current study.

Pediatrics
The safety and effectiveness of Noxafil injection in pediatric patients below the age of 18 years of age has not been established. Subjects <18 years of age will be excluded from the current study.

Geriatric Use
Of the 230 patients treated with posaconazole delayed-release tablets, 38 (17%) were greater than 65 years of age. The pharmacokinetics of posaconazole delayed-release tablets are comparable in young and elderly subjects. No overall differences in safety were observed between the geriatric patients and younger patients; therefore, no dosage adjustment is recommended for geriatric patients. No overall differences in the pharmacokinetics and safety were observed between elderly and young subjects during clinical trials,

Gender
The pharmacokinetics of posaconazole are comparable in men and women.

Weight
Pharmacokinetic modeling suggests that patients weighing greater than 120 kg may have lower posaconazole plasma drug exposure.

Renal Impairment
Following single-dose administration of 400 mg of the oral suspension, there was no significant effect of mild (eGFR: 50-80 mL/min/1.73 m_2, n=6) or moderate (eGFR: 20-49 mL/min/1.73 m_2, n=6) renal impairment on posaconazole pharmacokinetics; therefore, no dose adjustment is required in patients with mild to moderate renal impairment. In subjects with severe renal impairment (eGFR: <20 mL/min/1.73 m_2), the mean plasma exposure (AUC) was similar to that in patients with normal renal function (eGFR: >80 mL/min/1.73 m_2); however, the range of the AUC estimates was highly variable (CV=96%) in these subjects with severe renal impairment as compared to that in the other renal impairment groups (CV<40%). Due to the variability in exposure, patients with severe renal impairment being treated for fungal infections should be monitored closely for breakthrough fungal infections, although this recommendation is not relevant to the present trial. Similar recommendations apply to posaconazole delayed-release tablets; however, a specific study has not been conducted with the delayed-release tablets.

Hepatic Impairment
After a single oral dose of posaconazole oral suspension 400 mg, the mean AUC was 43%, 27%, and 21% higher in subjects with mild (Child-Pugh Class A, N=6), moderate (Child-Pugh Class B, N=6), or severe (Child-Pugh Class C, N=6) hepatic impairment, respectively, compared to subjects with normal hepatic function (N=18). Compared to subjects with normal

hepatic function, the mean C_{max} was 1% higher, 40% higher, and 34% lower in subjects with mild, moderate, or severe hepatic impairment, respectively. The mean apparent oral clearance (CL/F) was reduced by 18%, 36%, and 28% in subjects with mild, moderate, or severe hepatic impairment, respectively, compared to subjects with normal hepatic function. The elimination half-life ($t_{½}$) was 27 hours, 39 hours, 27 hours, and 43 hours in subjects with normal hepatic function and mild, moderate, or severe hepatic impairment, respectively. It is recommended that no dose adjustment of Noxafil is needed in patients with mild to severe hepatic impairment (Child-Pugh Class A, B, or C). Similar recommendations apply to posaconazole delayed-release tablets; however, a specific study has not been conducted with the delayed-release tablets.

OVERDOSAGE
There is no experience with overdosage of posaconazole injection and delayed-release tablets. During the clinical trials, some patients received posaconazole oral suspension up to 1600 mg/day with no adverse reactions noted that were different from the lower doses. In addition, accidental overdose was noted in one patient who took 1200 mg BID posaconazole oral suspension for 3 days. No related adverse reactions were noted by the investigator. Posaconazole is not removed by hemodialysis.

2.3 Clinical Data to Date

2.3.1 Safety data from placebo-controlled trials of oral delayed-release tablets

The safety of posaconazole delayed-release tablets has been assessed in 230 patients in clinical trials. Patients were enrolled in a non-comparative pharmacokinetic and safety trial of posaconazole delayed-release tablets when given as antifungal prophylaxis (Delayed-Release Tablet Study 1). Patients were immunocompromised with underlying conditions including hematological malignancy, neutropenia post-chemotherapy, GVHD, and post HSCT. This patient population was 62% male, had a mean age of 51 years (range 19-78 years, 17% of patients were ≥65 years of age), and were 93% white and 16% Hispanic. Posaconazole therapy was given for a median duration of 28 days. Twenty patients received 200 mg daily dose and 210 patients received 300 mg daily dose (following twice daily dosing on Day 1 in each cohort). Table below presents treatment-emergent adverse reactions observed in patients treated with 300 mg daily dose at an incidence of ≥10% in posaconazole delayed-release tablet study. The most frequently reported adverse reactions (>25%) with posaconazole delayed-release tablets 300 mg once daily were diarrhea, pyrexia, and nausea. The most common adverse reaction leading to discontinuation of posaconazole delayed-release tablets 300 mg once daily was nausea (2%).

Table: Posaconazole Delayed-Release Tablet Study 1: Number (%) of Subjects Treated with 300 mg Daily Dose Reporting Treatment-Emergent Adverse Reactions: Frequency of at Least 10%

	Posaconazole delayed-release tablet (300 mg) (n=210)	
Subjects reporting any Adverse Reaction	**201**	**(99)**
Anemia	22	(10)
Thrombocytopenia	29	(14)
Abdominal Pain	23	(11)
Constipation	20	(10)
Diarrhea	61	(29)
Nausea	56	(27)
Vomiting	28	(13)

Asthenia	20	(10)
Chills	22	(10)
Mucosal inflammation	29	(14)
Edema Peripheral	33	(16)
Pyrexia	59	(28)
Hypokalemia	46	(22)
Hypomagnesemia	20	(10)
Headache	30	(14)
Rash	34	(16)
Hypertension	23	(11)

CONTRAINDICATIONS
Hypersensitivity: Posaconazole is contraindicated in persons with known hypersensitivity to posaconazole or other azole antifungal agents.

QT Prolongation with Concomitant Use with CYP3A4 Substrates: Posaconazole is contraindicated with CYP3A4 substrates that prolong the QT interval. Concomitant administration of posaconazole with substrates pimozide and quinidine may result in increased plasma concentrations of these drugs, leading to QTc prolongation and torsades de pointes.

Coadministration with the HMG-CoA Reductase Inhibitors Primarily Metabolized Through CYP3A4: HMG-CoA reductase inhibitors (e.g., atorvastatin, lovastatin, and simvastatin) are contraindicated due to increased plasma concentration of these drugs can lead to rhabdomyolysis.

Ergot Alkaloids: Posaconazole may increase the plasma concentrations of ergot alkaloids (ergotamine and dihydroergotamine) which may lead to ergotism.

2.4 Dose Rationale

Noxafil® delayed-release tablets are available as yellow, coated, oblong tablets, debossed with "100" on one side containing 100 mg of posaconazole. Noxifil® delayed-release tablets are approved for prophylaxis of invasive aspergillus and Candida infection at a dose of 300mg (3 tablets) twice a day on the first day (loading dose) followed by 300mg (3 tablets) once a day, starting on the second day (maintenance dose). At this dose, steady state parameters are as noted in Table 1. The current trial will evaluate the effects of this FDA-approved dose (for fungal prophylaxis) in patients with Crohn's disease demonstrating genetic susceptibility to fungal infection. We propose a 3 month treatment duration which is expected to have inhibitory effects on the intestinal fungome (specifically *Malassezia* spp) as well as clinical effects on inflammation, as evidenced by a case series demonstrating clinical benefit for 5 patients with Crohn's disease after itraconazole therapy was incidentally prescribed for fungal infections.[34]

3 Study Objectives

Primary Objective

- To assess the effects of posaconazole endoscopic disease activity at week 12 in patients with active Crohn's disease

Secondary Objectives

- To assess the preliminary efficacy of posaconazole for the induction of clinical remission in patients with active Crohn's disease with genetically-determined CARD9 S12N risk allele

- To assess the preliminary safety and tolerability of posaconazole for the induction of clinical remission and endoscopic response in patients with active Crohn's disease with genetically-determined CARD9 S12N risk allele

- To assess the preliminary influence on quality-of-life of posaconazole in patients with active Crohn's disease with genetically-determined CARD9 S12N risk allele

- To assess the pharmacokinetics (PK) of posaconazole in patients with active Crohn's disease with genetically-determined CARD9 S12N risk allele

- To assess changes in the intestinal microbiota after treatment with posaconazole in patients with active Crohn's disease with genetically-determined CARD9 S12N risk allele

- To determine changes in gene expression (transcriptomics) in colonic mucosa after posaconazole in patients with active Crohn's disease with genetically-determined CARD9 S12N risk allele

4 Study Design

4.1 General Design

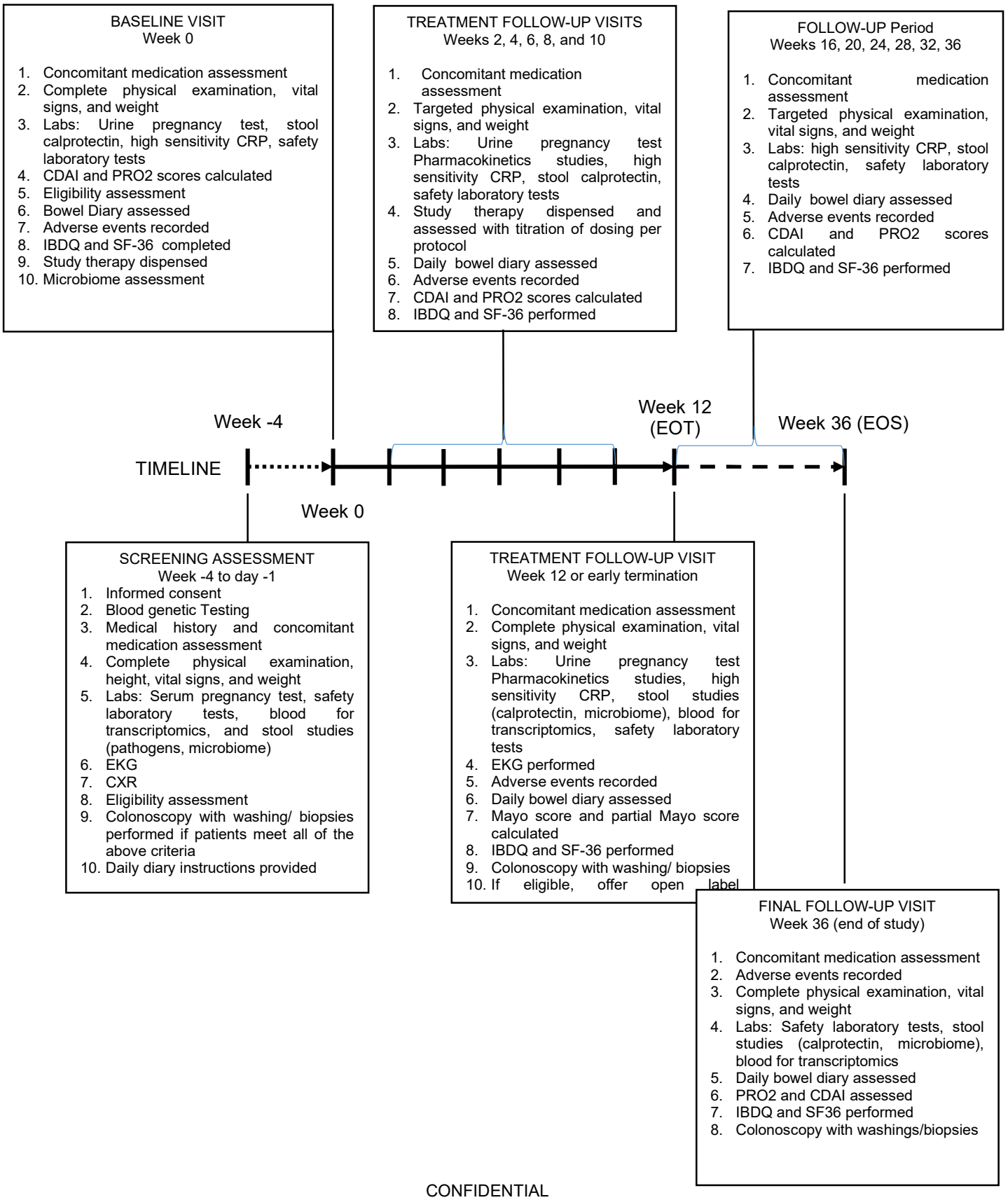
This study is designed as a two-center, placebo-controlled induction trial in 24 subjects with active Crohn's disease, who demonstrate at least one CARD9 S12N risk allele. Expected duration of participation is forty weeks. This will include screening period up to four-weeks and a twelve-week, double-blind induction period.

- Subjects who complete the 12-week induction period, meet criteria for active disease, AND upon unblinding at 12 weeks are determined to have received placebo during the 12-week induction period will be eligible to receive open-label posaconazole for 12 weeks, followed by a 12-week follow-up period.

- Subjects who complete the 12-week induction period and do not meet criteria for active disease, OR upon unblinding at 12 weeks are determined to have received posaconazole during the 12-week induction period will have a follow-up period of twenty-four weeks.

Clinical remission will be defined as the proportion of subjects with endoscopic improvement, defined by a decrease in 50% of the SES-CD endoscopic score. Subjects experiencing early withdrawal from study will be scheduled for a safety follow-up visit four weeks after the date of withdrawal.

Figure 1: Study Design



4.2 Primary Study Endpoints

The primary endpoint is the proportion of subjects in endoscopic response at week 12, defined by SES-CD reduction of >50% from baseline.

4.3 Key Secondary Study Endpoints

- The proportion of subjects in clinical remission at Week 12, defined by PRO2 (abdominal pain score ≤1 AND stool frequency ≤3) and by the Crohn's Disease Activity Index (<150)
- Reduction of Malassezia spp in colonic mucosal washings at week 12
- The safety of posaconazole, determined by assessment of AEs and concomitant medications throughout the duration of the study. The proportion of subjects in clinical remission at Week 8, defined by Mayo score≤2 with no subscore>1

4.4 Primary Safety Endpoints

- Incidence and severity of adverse events
- Change from baseline of laboratory values (chemistry, hematology, electrolytes, liver enzymes, renal function)
- Incidence of electrocardiogram (EKG) abnormalities including QTc prolongation

4.5 Quality-of-life Endpoints

- Inflammatory bowel disease questionnaire (IBDQ)
- Short Form SF-36 (SF-36)

4.6 Pharmacokinetics Endpoints

- Serum posaconazole concentrations at weeks 2, 4, 6, 8, 10, and 12.Relationship between posaconazole serum concentration and clinical and antifungal effects

4.7 Transcriptomics Endpoints

- Changes in stool and mucosal washing metabolites
- Changes in microbiome / metabolome reflected in gene expression patterns in mucosal biopsies

5 Subject Selection and Withdrawal

Subjects (n=24) will be individuals with active Crohn's disease (SES-CD ≥ 6 AND PRO2 score of abdominal pain ≥2 OR PRO2 daily stool frequency ≥ 4) despite use of conventional therapy. Subjects will be randomly assigned to receive posaconazole or placebo in 1:1 fashion. In this 40-week trial, subjects assigned to treatment will be administered posaconazole (Noxifil®, Merck) 300mg daily for 12 weeks. and then observed off posaconazole over the next 24 weeks. In addition to the treatment intervention, subjects also will provide samples for parallel translational studies including blood for genetic analysis, stool samples for biomarker and microbial composition analysis, and biopsies for mucosal gene expression analysis. Patients meeting criteria for active disease at 12 weeks will be offered open-label posaconazole for 12 weeks and will continue all study-related visits and procedures until final safety visit at week 36.

5.1 Randomization

At the baseline visit, subjects will be randomized via REDCap to receive posaconazole or placebo.

5.2 Inclusion Criteria

1. Male or female patients ≥ 18 years of age.
2. A diagnosis of CD with minimum disease duration of 6 months with involvement of the ileum and/or colon documented on colonoscopy
3. Have an endoscopically-confirmed active Crohn's disease with active disease defined by SES-CD ≥ 6 (≥4 if ileal only), AND active symptoms of Crohn's disease (CDAI >220)
4. Subjects receiving oral aminosalicylates (at a stable dose for 2 weeks prior to baseline), immunomodulators (at a stable dose for 4 weeks prior to baseline), anti-TNF, anti IL12/23, or anti-integrin therapy (at stable maintenance doses for ≥ 8 weeks) may continue their use during the study.
5. Subjects receiving oral corticosteroids may continue their use during the study provided the dose (prednisone up to 20 mg/day, budesonide up to 9 mg/day) has been stable for two weeks prior to screening.
6. Lab findings of hypokalemia, hypomagnesemia, or hypocalcemia are eligible, provided electrolyte abnormalities are corrected and stable prior to study enrollment.
7. Have had age-appropriate and disease-duration-appropriate colon cancer screening without unresected dysplasia.
8. Women of childbearing age, excluding those with prior bilateral tubal ligation or at least one-year post-menopause, must not be pregnant, lactating, or planning to become pregnant. They must agree to use effective contraception throughout the study period.
    a. Option 1: Any one of the following highly effective methods: hormonal contraception for example, birth control pills, injection, implant, transdermal patch, vaginal ring); intrauterine device (IUD); tubal ligation (tying your tubes); or a partner with a vasectomy
    b. Option 2: Any two of the following effective methods: male or female condom PLUS one of the following additional barrier methods (a)diaphragm with spermicide; (b) cervical cap with spermicide; or (c) contraceptive sponge with spermicide
9. Subjects must be able to provide informed consent and understand, agree with, and be able to adhere to daily diary entries, all scheduled visits, tests, procedures, and protocol in English.

5.3 Exclusion Criteria

The presence of any of the following will exclude a subject from enrollment:

1. Known hypersensitivity or allergy to posaconazole or other azole antifungal agents
2. Concomitant medications primarily metabolized by CYP3A4 including (***but not limited to***):
    a. HMG-CoA inhibitors primarily metabolized by CYP3A4 (increases risk of rhabdomyolysis)
    b. Sirolimus
    c. Ergot alkaloids
    d. Vincristine
    e. upadacitinib
3. Proarrhythmic conditions
4. Moderate or severe renal impairment (Cr Clearance <50)
5. Elevated transaminases or bilirubin > 2.5x ULN.
6. Current diagnosis of ulcerative colitis, indeterminate colitis, ischemic colitis, infectious colitis, or microscopic colitis.
7. Fulminant colitis, toxic megacolon, peritonitis, ileostomy or colostomy.

8. Stool sample positive for pathogens including ova and parasites, *Salmonella*, *Shigella*, and *C. difficile* at screening.
9. History of any clinically significant neurological, renal, hepatic, gastrointestinal, pulmonary, metabolic, cardiovascular, psychiatric, endocrine, hematological disorder or disease or any other medical condition that, in the Investigators opinion, would prevent the subject from participation in the study.
10. Treatment with antibiotics, antifungal agents, probiotics, or prebiotics within two weeks of screening.
11. Alcohol or drug abuse (in the opinion of the Investigator) that would interfere with compliance.

5.4 Subject Recruitment and Screening

Potential study candidates will be identified from the Cedars-Sinai Inflammatory Bowel Disease Center genetic database (MIRIAD) and Mayo Clinic's IBD Biobank; all MIRIAD and Biobank participants have previously provided written, informed consent to participate in genetic research and to be contacted for potential research opportunities. Patients who have CARD9 risk allele will be notified of an opportunity to participate in this trial at the time of a routine clinical encounter, if deemed appropriate by their treating provider. They will be provided with information about the study, and if the study candidate is interested in study participation, they will be scheduled for the screening visit.

Patients who wish to participate in this research, who have not previously had their CARD9 risk allele testing performed or who are not in MIRIAD or Mayo Clinic's IBD Biobank, will have an opportunity to provide a blood sample for genetic testing of CARD9, once Informed Consent to participate has been provided.

At the screening visit, patients will be asked to provide informed consent for the study. A complete medical history will be taken including list of current and recent medications. A complete physical examination, including height, weight, vital signs will be performed. Safety laboratory studies, pregnancy test, stool studies, and EKG will be performed at this visit (stool may be provided after the visit). Chest radiography will be performed. Then, patients meeting eligibility will be scheduled for baseline colonoscopy. Patients meeting eligibility criteria will be scheduled for the baseline visit.

5.5 *Lifestyle guidelines*

Patients will be instructed to continue their current dietary and exercise patterns during the trial, excluding non-prescription supplements or herbal preparations which might affect hepatic metabolism.

5.6 Early withdrawal of Subjects

5.6.1 When and How to Withdraw Subjects

Subjects may be withdrawn from study for safety concerns, progression of Crohn's disease (requiring new medication or escalation of dose of current therapies, hospitalization, or surgery), withdrawal of consent, failure to adhere to protocol requirements, patient request, or administrative reasons. Withdrawal may be warranted at the request of the patient, investigator, or study protocol. The reason for study withdrawal will be determined by the investigator. Patients will be required to return all remaining study therapy.

The data from subjects who withdraw consent will continue to be used in analysis before the date of withdrawal of consent, however no follow-up or inquiries will be conducted on these patients on or after this date.

If a subject does not attend a scheduled visit, the subject will be called three times at the number provided by the subject. Subjects who do not answer will be sent a certified letter. The record of these contacts and any written communication will be included in the study documentation. Subjects who do not respond to these inquiries will be assigned as lost to follow-up (LTFU).

5.6.2 Data Collection and Follow-up for Withdrawn Subjects

Subjects with early withdrawal from study during the twelve-week intervention period, for any reason, will be scheduled for a safety follow-up visit four weeks from date of withdrawal. Subjects who withdraw from study after the intervention period will maintain the per-protocol final follow-up visit at week 36. The clinical outcome will be assessed at this follow-up visit.

Subjects with abnormal laboratory findings, or other adverse events requiring early study withdrawal, will be followed until the abnormality has resolved to baseline or steady state. The investigators will determine if the adverse event was related to study therapy. The clinical course will be documented.

5.7 Open Label Posaconazole:

At 12 weeks, subjects will undergo colonoscopy and clinical assessment for response, after which they will be unblinded. Subjects who meet criteria for active disease AND who are determined to have received placebo will be eligible to receive 12 weeks of open-label posaconazole. Eligibility Criteria for open label posaconazole:

1. Endoscopically-confirmed active Crohn's disease with active disease defined by SES-CD $\geq$ 6 ($\geq$4 if ileal only) based on local reading, AND
2. Active symptoms of Crohn's disease (CDAI >220), AND
3. Received placebo during the 12-week induction period

Subjects who meet the above criteria and opt to receive open label posaconazole will be monitored for safety / adverse events at follow-up visits. Changes in corticosteroid dosing will be permitted after 12 weeks, regardless of whether subjects are receiving posaconazole or not. All subjects will undergo colonoscopy at 36 weeks.

6 Study Drug

6.1 Description

Noxafil delayed-release tablets are available as yellow, coated, oblong tablets, debossed with "100" on one side containing 100 mg of posaconazole.

6.2 Treatment Regimen

Posaconazole (Noxifil®) delayed-release tablets: Loading dose: 300mg (three 100mg delayed-release over-encapsulated tablets) twice a day on the first day, then Maintenance dose: 300mg (three 100mg delayed-release over-encapsulated tablets) once a day, starting on the second day. Duration of therapy will be 12 weeks.

6.3 Preparation and Administration of Study Drug

To maintain study blind, placebo capsules will be manufactured using blue gelatin capsules, size 00, and filled with Cellulose Microcrystalline. Posaconazole tablets will be over-encapsulated, using the same blue gelatin capsules, size 00, and back-filled with Cellulose Microcrystalline.

Posaconazole will be self-administered by the subjects, with the first dose observed at the time of the baseline visit. Subjects will be advised to swallow the capsules whole, and to not divide, crush or chew. The capsules should be taken with food to enhance the oral absorption of posaconazole and optimize plasma concentrations.

6.4 Subject Compliance Monitoring

Subjects need to keep the capsule containers and bring them to follow up visit. Compliance will be evaluated by the counts of unused tablets. If a deviation from protocol is identified, the investigators will inquire as to the reason for study deviation during the corresponding patient encounter. Non-compliance related to study medication regimen and (i.e., less than 80% or more than 120% of recommended dosing) and not due to adverse events will be evaluated by the Investigator who will encourage subject to follow the medication regimen prescribed. Patients who remain significantly non-compliant with the drug regimen and not due to adverse events for two consecutive visits will be withdrawn from study or earlier at the discretion of the PI. The subject will then complete the final follow-up visit per protocol.

6.5 Prior and Concomitant Therapy

The following concomitant therapies are allowed as long as a stable dose was administered per eligibility criteria:

- Biologic agents (infliximab, adalimumab, certolizumab, ustekinumab, vedolizumab)
- Immunomodulators (azathioprine/6-mercaptopurine)
- Oral 5-aminosalicylates or sulfasalazine

Oral corticosteroid preparations (maximum budesonide 9 mg/day, or prednisone 20 mg/day or equivalent) will be allowed as long as the dose has been stable dose for two weeks prior to screening. The dosage may not be reduced during the 12-week study period without premature discontinuation from the trial. Any dosage increases beyond that prescribed at the start of the trial will result in early withdrawal from study due to progression of Crohn's disease.

Concomitant prescribed therapies, non-prescription supplements, and herbal preparations which affect hepatic metabolism, antibiotics, probiotics, and antidiarrheals will not be allowed during the study. Drugs predominantly metabolized by CYP3A4 may be increased by posaconazole. Examples of drugs prohibited during the current proposed trial include, but are not limited to: cyclosporine, tacrolimus, sirolimus, simvastatin and other HMG-CoA inhibitors, midazolam, rifabutin, phenytoin, ritonavir, atazanavir, and upadacitinib.

Data regarding the composition, dosage, dosing schedule, route of administration, and initiation/end dates of therapy will be recorded for each concomitant therapy.

6.6 Packaging

Noxafil delayed-release tablets are available as yellow, coated, oblong, debossed with "100" on one side containing 100 mg of posaconazole. Bottles with child-resistant closures of 60 delayed-

release tablets (NDC 0085-4324-02). Upon arrival at site, tablets will be over-encapsulated with blue gelatin capsules.

6.7 Receiving, Storage, Dispensing and Return

Store at 20-25°C (68-77°F), excursions permitted to 15-30°C (59-86°F) [see USP Controlled Room Temperature].

6.7.1 Receipt of Drug Supplies

Upon receipt of the of the study treatment supplies, an inventory will be performed, and a drug receipt log filled out and signed by the person accepting the shipment. Designated study staff will verify that the shipment contains all the items noted in the shipment inventory. Any damaged or unusable study drug in a given shipment will be documented in the study files.

6.7.2 Storage

Store at 20-25°C (68-77°F), excursions permitted to 15-30°C (59-86°F)

6.7.3 Dispensing of Study Drug

Patients will be dispensed a 2-week supply of study drug (or placebo) at each of the following study visits: baseline (week 0), and weeks 2, 4, 6, 8, and 10. Study drug reconciliation will be performed to document drug assigned, drug consumed, and drug remaining. This reconciliation will be logged on the drug reconciliation form and signed and dated by the study team.

Patients who are eligible to receive open label posaconazole will be dispensed a 4-week supply of study drug at weeks 12, 16, and 20.

6.7.4 Return or Destruction of Study Drug

At the completion of the study, there will be a final reconciliation of drug shipped, drug consumed, and drug remaining. This reconciliation will be logged on the drug reconciliation form, signed and dated. Any discrepancies noted will be investigated, resolved, and documented prior to return or destruction of unused study drug. Drug will be destroyed per research pharmacy discretion.

6.8 Placebo

Placebo capsules will be developed for this study and will duplicate the size, color and shape of the over-encapsulated Noxifil®.

7 Study Procedures

7.1 Screening (Week -4 to Week 0)

After informed consent is given, eligibility will be evaluated over a period of four weeks. The study investigators will discuss with the patient the purpose, obligations, and risks of the study.

The following tasks will be conducted or scheduled at the initial Week -4 visit:

- Complete medical history: date of diagnosis; extent of disease; date and reason for CD-related hospitalizations; past medical history (including prior invasive fungal infection, arrythmia, chronic kidney disease stage III or greater, uncontrolled diabetes mellitus, cirrhosis, and major psychiatric disorder); alcohol and drug history; and extra-intestinal manifestations.

- Medication history: list of current medications including date of initiation, length of current dosing regimen, current dose, current frequency, current route of administration; list of prior medications, including clinical response to medication and reason for medication withdrawal; all non-prescription medications, non-prescription supplements, and herbal preparations used within last twelve weeks.
- Complete physical examination, height, vital signs, and weight.
- Laboratory studies: serum β-hCG in women of childbearing potential, safety laboratory tests (serum chemistries, complete blood count with automatic differential, lipid panel, hemoglobin A1c, urinalysis), and stool studies (stool ova and parasitology, culture, and C. difficile toxin assay,
- 12-lead EKG.
- Chest radiography posteroanterior and lateral (if patient meets all of the above eligibility requirements) if not performed within last 12 months.
- Bowel movement diary instructions.
- Eligibility assessment based on collected data.
- Colonoscopy to be scheduled within one week of baseline visit, with SES-CD score
    - Mucosal biopsies will be collected from the Ileum, Right colon, and Left Colon. 2 biopsies from most inflamed area of each region will be taken. One tissue will be placed in a solution of RNALater, and the other tissue will be placed in Formalin.
    - and Washings for fungome analyses
- Stool collection for microbiome analysis, stored at -70C, and batch shipped every 6-Months.
- Blood for transcriptomics.
- Blood for genetics (if CARD9 status is not known)

7.2 Baseline visit (week 0)

The following tasks will be conducted at this visit:

- Assessment of concomitant medications.
- Complete physical examination, vital signs, and weight.
- Laboratory studies: urine pregnancy test in women of childbearing potential, hsCRP, serum chemistries, complete blood count with automatic differential, lipid panel, and stool calprotectin.
- Calculation of CDAI and PRO2 scores
- Eligibility assessment based on collected data.
- Study therapy dispensed and instructions on use given.
- Adverse event monitoring.
- Review of bowel movement diary.
- IBDQ and SF-36 assessments.
- Randomization of subjects to treatment arm via REDCap by research pharmacist. (Both subject and clinical staff will be masked to treatment group assigned.)

7.3 Weeks 2, 4, 6, 8, and 10 (±3 days)

These visits may be conducted within three days of the specific time-point marking the number of weeks since baseline visit. The following tasks will be conducted at these visits:

- Assessment of concomitant medications.
- Targeted physical examination (eyes, throat, lymph nodes, cardiopulmonary systems, abdomen, musculoskeletal, extremities, and skin), vital signs, and weight.

- Laboratory studies: urine pregnancy test in women of childbearing potential, serum posaconazole concentration, hsCRP, serum chemistries, lipid panel, complete blood count with automatic differential, and stool calprotectin.
- Study therapy usage reviewed, and further study drug dispensed
- Adverse event monitoring.
- Review of bowel movement diary.
- Calculation of CDAI and PRO2 scores
- IBDQ and SF-36 assessments.
- Stool collection for microbiome analysis

7.4 Week 12 (±3 days) or early termination

This visit may be conducted within three days of the specific time-point marking the number of weeks since baseline visit. The following tasks will be conducted at these visits:

- Assessment of concomitant medications.
- Complete physical examination, vital signs, and weight.
- Laboratory studies: urine pregnancy test in women of childbearing potential, serum posaconazole concentration, hsCRP, serum chemistries, complete blood count with automatic differential, lipid panel, and stool calprotectin, microbiome, blood for transcriptomics.
- 12-lead EKG.
- Study therapy usage reviewed.
- Adverse event monitoring.
- Review of bowel movement diary.
- Calculation of CDAI and PRO2 scores
- IBDQ and SF-36 assessments.
- Colonoscopy with biopsies (RNALater & Formalin) and washings scheduled ±7 days of this visit for calculation of SES-CD. Biopsies and washings should be obtained from same area of each region as screening samples.
- Following the above evaluations, subjects will be unblinded and determined to have received either posaconazole or placebo during the 12-week induction period.
- Patients meeting criteria for active disease AND who are determined to have received placebo will be eligible to receive open label posaconazole; these subjects will be dispensed 4 weeks posaconazole at this visit.

7.5 Weeks 16, 20, 24, 28, 32 (±3 days)

This visit may be conducted within three days of the specific time-point marking the number of weeks since baseline visit. The following tasks will be conducted at these visits:

- Assessment of concomitant medications.
- Targeted physical examination, vital signs, and weight.
- Laboratory studies: serum chemistries, complete blood count with automatic differential, lipid panel, and stool calprotectin and microbiome (not collected at W28 & 32), blood for transcriptomics
- Review of bowel movement diary.
- Calculation of CDAI and PRO2 scores
- IBDQ and SF36 assessments
- Adverse event monitoring.

- Weeks 16, 20, and 24: Female subjects receiving open label posaconazole will undergo urine pregnancy test, and all subjects receiving open label posaconazole will undergo EKG.

7.6 Week 36 visit (or final follow up visit after early termination)

The following tasks will be conducted at this visit:

- Assessment of concomitant medications.
- Complete physical examination, vital signs, and weight.
- Laboratory studies: urine pregnancy test in women of childbearing potential, hsCRP, serum chemistries, complete blood count with automatic differential, lipid panel, and stool calprotectin.
- Calculation of CDAI and PRO2 scores
- Eligibility assessment based on collected data.
- Adverse event monitoring.
- Review of bowel movement diary.
- IBDQ and SF-36 assessments.
- Colonoscopy with biopsies (RNALater & Formalin) and washings scheduled ±7 days of this visit for calculation of SES-CD. Biopsies & washings should be obtained from same area of each region as screening samples.

8 Assessments

8.1 Efficacy measures

8.1.1 Bowel movement diary

Subjects will be provided with a daily diary to record bowel movement frequency and abdominal pain. Subjects will record the usual number of stools per day when not having a flare. Within their daily bowel movement history, subjects will record the number of bowel movements per day, and presence of blood in stool.

8.1.2 Crohn's disease activity Index (CDAI), PRO2 and Simple Endoscopic Score for Crohn's disease (SES-CD)

These scoring systems assess Crohn's disease activity. The CDAI score ranges from 0 to 600, and has traditionally been used to assess clinical disease activity for Crohn's disease clinical trials. Clinical remission will be defined by a CDAI score of <150 points AND a patient-reported outcome 2-item (PRO2) score of abdominal pain score $\leq$1 AND stool frequency $\leq$3. (See Appendix for calculations of both CDAI and PRO2). The SES-CD will determine endoscopic activity for eligibility as well as for determining mucosal improvement (See Appendix for SES-CD calculation).

8.1.3 Health-related quality of life

The following quality-of-life measures are recorded by subjects at the corresponding visits (pending permissions):

- Inflammatory bowel disease questionnaire (IBDQ)
- Short Form SF-36 (SF-36)

8.2 Laboratory markers

- Serum chemistry (sodium, potassium, chloride, magnesium, phosphorous, bicarbonate, blood urea nitrogen, creatinine, glucose, total protein, albumin, alanine aminotransferase, aspartate

aminotransferase, total bilirubin, direct bilirubin, alkaline phosphatase, gamma-glutamine transferase,)
- Complete blood count with automated differential (hemoglobin, hematocrit, white blood cell count, platelet count)
- Urinalysis (pH, color, blood, specific gravity, glucose, protein, leukocyte esterase, nitrite) with microscopy if presence of blood, leukocyte esterase, nitrite, or if there is a clinical indication
- Serum β-hCG
- Urine β-hCG
- High-sensitivity C-reactive protein (hsCRP)
- Lipids
- Stool tests for C difficile, enteric pathogens, calprotectin

8.3 Pharmacokinetics laboratory markers

- Serum posaconazole will be assessed at weeks 2, 4, 6, 8, 10, and 12. The exact time of day and date of collection will be recorded.

8.4 Imaging and cardiac studies

- Chest xray (posteroanterior and lateral): findings must not be consistent with infiltrate, pulmonary malignancy, heart failure, or respiratory disease.
- 12-lead EKG: findings must not be consistent with ischemia or prior infarction, left ventricular hypertrophy, delayed conduction, prolonged QT (>450ms in male or >460ms in female), or serious arrhythmias.

8.5 Microbiome analyses

Stool for microbiome analysis will be collected at screening visit and weeks 0, 2, 4, 6, 8, 10, 12, 16, 20, 24, 36 or early termination. Microbial community structure will be analyzed from stored fecal samples collected in each patient group. Samples will be kept at -80 C until DNA extraction. The V4 region of the 16S rRNA gene will be PCR amplified in triplicate, and the resulting amplicons cleaned, quantified and sequenced on the Illumina MiSeq platform using custom barcoded primers. Raw sequences will be processed using the QIIME software package (Quantitative Insights Into Microbial Ecology; http://qiime.org/). Only full-length, high-quality reads will be used for analysis. OTUs are picked at 97% similarity against the Greengenes database. Subsequent analysis of 16S rRNA data will include weighted and un-weighted UniFrac distance matrix, and calculations of α and β diversity.

8.6 Transcriptomics analyses

Serum metabolites and colonic mucosa will be obtained at Screen, week 12, and week 24 Blood and colonic mucosa (screening, week 12, week 36) will be collected for analyzing serum metabolites and RNAseq to determine treatment-related changes in gene expression (transcriptomics).

9 Statistical Plan

9.1 Sample Size Determination

The primary endpoint is endoscopic response, defined as a 50% reduction SES-CD scores at week 12. We will assume a placebo response rate of 10% [2-4], and a treatment response rate of 70%. Therefore, with a study size of 24 patients (11 in each group, plus 2 potential dropouts before the second colonoscopy) there will be 76% power in a Fisher's Exact test with a target

alpha of 0.05. The significance level actually achieved with binomial enumeration is 0.002. (Power estimates computed with PASS2020 software).

9.2 Statistical Methods

Secondary endpoints will include clinical remission (defined as Crohn's disease activity index < 150), analysis of data regarding use of corticosteroids, mucosal healing, mucosal improvement, normalization of inflammatory biomarkers, and improvement in health-related quality of life. Repeated measures analysis will be used to examine changes over time from baseline. Categorical data will be analyzed with repeated measures logistic regression while continuous measures will used mixed modeling regression. Residuals will be inspected to confirm data meets assumptions necessary for parametric testing. Data will be considered significant at the two-sided p-value of <0.05. All analysis will be performed by the study biostatistician (C Bresee, MS) using SAS v9.4 software.

- Primary Study Endpoints: The proportion of subjects with endoscopic response defined by a 50% reduction in SES-CD score at 12 weeks
- Key Secondary Study Endpoints: The proportion of subjects in clinical remission at Week 12
- Primary Safety Endpoints: Incidence and severity of adverse events, change from baseline of laboratory values (chemistry, hematology), incidence of electrocardiogram (EKG) abnormalities
- Quality-of-life Endpoints: Change from baseline in Inflammatory bowel disease questionnaire (IBDQ) and Short Form SF-36 (SF-36).
- Pharmacokinetic Endpoints: Changes in dosing regimen during study, relationship between nicotine concentrations and clinical effects.
- Intestinal Microbiota Endpoints: Subsequent analysis of 16S rRNA data will include weighted and un-weighted UniFrac distance matrix, and calculations of α and β diversity.
- Transcriptomics Endpoints: Our group has developed a standard transcriptome analysis pipeline, which uses the TopHat software package for performing gapped alignments against the reference genome, DESeq for detecting differential gene expression, and Cufflinks for detecting differential isoform expression.
  TopHat improves upon earlier RNA-Seq alignment tools as it allows paired-end sequencing, longer reads, and particularly gapped alignments of read fragments that reduce the uncertainty in assigning reads to alternative splice variants. DESeq uses a negative binomial distribution model for read counts to identify differential gene expression. Cufflinks uses a normal distribution model for the transcript length and linear model for the likelihood function to identify differentially expressed transcripts.
- Sample size: 12 patients will be recruited for this open-label pilot study. Given the pilot nature of this study, a sample size justification is not provided.

9.3 Subject Population(s) for Analysis

- Intention-to-treat (ITT) population: All subjects in the study who received at least one dose of study drug
- Per-protocol population: All subjects in the study who received the protocol-required study drug

10 Safety and Adverse Events

10.1 Recording of Adverse Events

At each contact with the subject, the investigator must seek information on adverse events by specific questioning and, as appropriate, by examination. Information on all adverse events should be recorded immediately in the source document. All clearly related signs, symptoms, and abnormal diagnostic procedures results should be recorded in the source document, though should be grouped under one diagnosis.

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

10.1.1 Investigator reporting: notifying the Cedars Sinai IRB

The Cedars Sinai IRB requires expedited reporting of those events related to study participation that are unforeseen and indicate that participants or others are at increased risk of harm. The Cedars Sinai IRB requires researchers to submit reports of the following problems within 10 working days from the time the investigator becomes aware of the event:

- Any adverse event (regardless of whether the event is serious or non-serious, on-site or off-site) that occurs any time during or after the research study, which in the opinion of the principal investigator is:

  Unexpected (An event is "unexpected" when its specificity and severity are not accurately reflected in the protocol-related documents, such as the IRB-approved research protocol, any applicable investigator brochure, and the current IRB-approved informed consent document and other relevant sources of information, such as product labeling and package inserts.)

  AND

  Related to the research procedures (An event is "related to the research procedures" if in the opinion of the principal investigator or sponsor, the event was more likely than not to be caused by the research procedures.)

Reporting Process

Unanticipated problems posing risks to subjects or others as noted above will be reported to the Cedars Sinai IRB using the form: "Unanticipated Problems Posing Risks to Subjects or Others Including Reportable Adverse Events" or as a written report of the event (including a description of the event with information regarding its fulfillment of the above criteria, follow-up/resolution and need for revision to consent form and/or other study documentation).

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

Other Reportable events:

For clinical drug trials, the following events are also reportable to the Cedars Sinai IRB:

- Any adverse experience that, even without detailed analysis, represents a serious unexpected adverse event that is rare in the absence of drug exposure (such as agranulocytosis, hepatic necrosis, Stevens-Johnson syndrome).

- Change in FDA safety labeling or withdrawal from marketing of a drug, device, or biologic used in a research protocol.
- Breach of confidentiality
- Change to the protocol taken without prior IRB review to eliminate apparent immediate hazard to a research participant.
- Incarceration of a participant when the research was not previously approved under Subpart C and the investigator believes it is in the best interest of the subject to remain on the study.
- Complaint of a participant when the complaint indicates unexpected risks, or the complaint cannot be resolved by the research team.
- Protocol violation (meaning an accidental or unintentional deviation from the IRB approved protocol) that in the opinion of the investigator placed one or more participants at increased risk or affects the rights or welfare of subjects.

10.2 Medical Monitoring

It is the responsibility of the Principal Investigator to oversee the safety of the study. This safety monitoring will include careful assessment and appropriate reporting of adverse events as noted above.

11 Data Handling and Record Keeping

11.1 Confidentiality

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

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

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

11.2 Source Documents

Source data is all information, original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Source data are contained in source documents. Examples of these original documents, and data records include: hospital records, clinical and office charts, laboratory notes, memoranda, subjects' diaries or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate and complete, microfiches, photographic negatives, microfilm or magnetic media, x-rays, subject files, and records kept at the pharmacy, at the laboratories, and at medico-technical departments involved in the clinical trial.

11.3 Records Retention

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

11.4 Sample Storage

For this study, subjects are being asked to let the Investigator use their blood and stool samples collected during the study for future research. Their samples will be labeled with a code. Their identity will be protected, and their name will not be attached to the samples. The samples will be prepared and stored in a freezer for up to 20 years. After 20 years, the samples will be destroyed. The stored samples may be used for gaining information about illness and to help answer study questions about the drug.

12 Study Monitoring, Auditing, and Inspecting

12.1 Study Monitoring Plan

This pilot, investigator-initiated study will be routinely monitored as outlined in monitoring plan provided by UCLA CTSI Office of Regulatory Affairs' (ORA) Quality Assurance Team (Appendix 6) .

12.2 Auditing and Inspecting

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

13 Ethical Considerations

This study is to be conducted according to US and international standards of Good Clinical Practice (FDA Title 21 part 312 and International Conference on Harmonization guidelines), applicable government regulations and Institutional research policies and procedures.

This protocol and any amendments will be submitted to the Cedars-Sinai Institutional Review Board (IRB) for formal approval of the study conduct. The decision of the IRB concerning the conduct of the study will be made in writing to the investigator.

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

14 Study Finances

14.1 Conflict of Interest

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

15 Publication Plan

It is the intention of the investigators to publish the results of this study after study completion.

16 References

1. Levin B, Lieberman DA, McFarland B, et al. Screening and surveillance for the early detection of colorectal cancer and adenomatous polyps, 2008: a joint guideline from the American Cancer Society, the US Multi-Society Task Force on Colorectal Cancer, and the American College of Radiology. Gastroenterology 2008;134:1570-95.
2. Vermeire S, Schreiber S, Petryka R, et al. Clinical remission in patients with moderate-to-severe Crohn's disease treated with filgotinib (the FITZROY study): results from a phase 2, double-blind, randomised, placebo-controlled trial. Lancet 2017;389:266-275.
3. Feagan BG, Sandborn WJ, D'Haens G, et al. Induction therapy with the selective interleukin-23 inhibitor risankizumab in patients with moderate-to-severe Crohn's disease: a randomised, double-blind, placebo-controlled phase 2 study. Lancet 2017;389:1699-1709.
4. Rutgeerts P, Gasink C, Chan D, et al. Efficacy of Ustekinumab for Inducing Endoscopic Healing in Patients With Crohn's Disease. Gastroenterology 2018;155:1045-1058.
5. Kaplan GG. The global burden of IBD: from 2015 to 2025. Nat Rev Gastroenterol Hepatol 2015;12:720-7.
6. Afzali A, Cross RK. Racial and Ethnic Minorities with Inflammatory Bowel Disease in the United States: A Systematic Review of Disease Characteristics and Differences. Inflamm Bowel Dis 2016;22:2023-40.
7. Mehta F. Report: economic implications of inflammatory bowel disease and its management. Am J Manag Care 2016;22:s51-60.
8. Spencer EA, Davis SM, Mack DR, et al. Serologic Reactivity Reflects Clinical Expression of Ulcerative Colitis in Children. Inflamm Bowel Dis 2018.
9. Dubinsky MC. What is the role of serological markers in the diagnosis of IBD? Inflamm Bowel Dis 2008;14 Suppl 2:S185-6.
10. Ferrante M, Vermeire S, Katsanos KH, et al. Predictors of early response to infliximab in patients with ulcerative colitis. Inflamm Bowel Dis 2007;13:123-8.
11. Taylor KD, Plevy SE, Yang H, et al. ANCA pattern and LTA haplotype relationship to clinical responses to anti-TNF antibody treatment in Crohn's disease. Gastroenterology 2001;120:1347-55.
12. Knox NC, Forbes JD, Van Domselaar G, et al. The Gut Microbiome as a Target for IBD Treatment: Are We There Yet? Curr Treat Options Gastroenterol 2019;17:115-126.
13. Levy AN, Allegretti JR. Insights into the role of fecal microbiota transplantation for the treatment of inflammatory bowel disease. Therap Adv Gastroenterol 2019;12:1756284819836893.
14. Moayyedi P, Surette MG, Kim PT, et al. Fecal Microbiota Transplantation Induces Remission in Patients With Active Ulcerative Colitis in a Randomized Controlled Trial. Gastroenterology 2015;149:102-109 e6.

15. Paramsothy S, Kamm MA, Kaakoush NO, et al. Multidonor intensive faecal microbiota transplantation for active ulcerative colitis: a randomised placebo-controlled trial. Lancet 2017;389:1218-1228.
16. Rossen NG, Fuentes S, van der Spek MJ, et al. Findings From a Randomized Controlled Trial of Fecal Transplantation for Patients With Ulcerative Colitis. Gastroenterology 2015;149:110-118 e4.
17. Costello SP, Hughes PA, Waters O, et al. Effect of Fecal Microbiota Transplantation on 8-Week Remission in Patients With Ulcerative Colitis: A Randomized Clinical Trial. JAMA 2019;321:156-164.
18. Khan KJ, Ullman TA, Ford AC, et al. Antibiotic therapy in inflammatory bowel disease: a systematic review and meta-analysis. Am J Gastroenterol 2011;106:661-73.
19. Iliev ID, Funari VA, Taylor KD, et al. Interactions between commensal fungi and the C-type lectin receptor Dectin-1 influence colitis. Science 2012;336:1314-7.
20. Limon JJ, Tang J, Li D, et al. Malassezia Is Associated with Crohn's Disease and Exacerbates Colitis in Mouse Models. Cell Host Microbe 2019;25:377-388 e6.
21. Beaudoin M, Goyette P, Boucher G, et al. Deep resequencing of GWAS loci identifies rare variants in CARD9, IL23R and RNF186 that are associated with ulcerative colitis. PLoS Genet 2013;9:e1003723.
22. Rivas MA, Beaudoin M, Gardet A, et al. Deep resequencing of GWAS loci identifies independent rare variants associated with inflammatory bowel disease. Nat Genet 2011;43:1066-73.
23. Zhernakova A, Festen EM, Franke L, et al. Genetic analysis of innate immunity in Crohn's disease and ulcerative colitis identifies two susceptibility loci harboring CARD9 and IL18RAP. Am J Hum Genet 2008;82:1202-10.
24. Limon JJ, Skalski JH, Underhill DM. Commensal Fungi in Health and Disease. Cell Host Microbe 2017;22:156-165.
25. Wheeler ML, Limon JJ, Underhill DM. Immunity to Commensal Fungi: Detente and Disease. Annu Rev Pathol 2017;12:359-385.
26. Cao Z, Conway KL, Heath RJ, et al. Ubiquitin Ligase TRIM62 Regulates CARD9-Mediated Anti-fungal Immunity and Intestinal Inflammation. Immunity 2015;43:715-26.
27. McGovern DP, Gardet A, Torkvist L, et al. Genome-wide association identifies multiple ulcerative colitis susceptibility loci. Nat Genet 2010;42:332-7.
28. Janse M, Lamberts LE, Franke L, et al. Three ulcerative colitis susceptibility loci are associated with primary sclerosing cholangitis and indicate a role for IL2, REL, and CARD9. Hepatology 2011;53:1977-85.
29. Pointon JJ, Harvey D, Karaderi T, et al. Elucidating the chromosome 9 association with AS; CARD9 is a candidate gene. Genes Immun 2010;11:490-6.
30. Kiryluk K, Li Y, Scolari F, et al. Discovery of new risk loci for IgA nephropathy implicates genes involved in immunity against intestinal pathogens. Nat Genet 2014;46:1187-96.
31. Thapa D, Lee JS, Park MA, et al. Inhibitory effects of clotrimazole on TNF-alpha-induced adhesion molecule expression and angiogenesis. Arch Pharm Res 2009;32:593-603.
32. Thapa D, Lee JS, Park SY, et al. Clotrimazole ameliorates intestinal inflammation and abnormal angiogenesis by inhibiting interleukin-8 expression through a nuclear factor-kappaB-dependent manner. J Pharmacol Exp Ther 2008;327:353-64.
33. Takahashi H, Abe M, Sugawara T, et al. Clotrimazole, an imidazole antimycotic, is a potent inhibitor of angiogenesis. Jpn J Cancer Res 1998;89:445-51.
34. Samuel S, Loftus EV, Jr., Sandborn WJ. The effects of itraconazole on inflammatory bowel disease activity in patients treated for histoplasmosis. Aliment Pharmacol Ther 2010;32:1207-9.
35. Biancone L, Petruzziello C, Orlando A, et al. Cancer in Crohn's Disease Patients Treated with Infliximab: A Long-term Multicenter Matched Pair Study. Inflammatory Bowel Diseases 2011;17:758-766.

Appendices

- CDAI
- PRO2
- SES-CD
- CTCAE Grading Scale for severity of adverse events
- IBDQ form
- SF36 form
- DSMB Monitoring Plan

Appendix 1. Crohn's Disease Activity Index (CDAI) and Patient Reported Outcomes – 2 items (PRO2)

The Crohn's Disease Activity Index (CDAI) will be calculated as follows:

Number	Variable	Description	Multiplier
1	Liquid or very soft stool	Daily stool count summed over 7 days	2
2	Abdominal Pain	Sum of 7 days of daily ratings as 0 = none, 1 = mild, 2 = moderate, 3 = severe	5
	General Wellbeing	Sum of 7 days of daily ratings as 0 = generally well, 1 = slightly below par, 2 = poor, 3 = very poor, 4 = terrible	7
	Complications	Number of listed complications: Arthritis or arthralgia, Erythema nodosum, pyoderma gangrenosum or apthous stomatitis, Iritis or uveitis, Anal fissures or fistulae or abscess, Other fistula Fever over 37.8 C [100 F] during past week	20 each
	Use of antidiarrheal medications	Use of diphenoxylate or loperamide or other opiate for diarrhea 0 = No, 1 = Yes	30
	Abdominal Mass	0 = none, 2 = questionable, 5 = definite	10
	Hematocrit	Males: 47 – Hct [%] Females: 42 – Hct [%] *Result must be greater than or equal to 0. If negative result enter 0	6x difference
	Weight	Percentage deviation from standard weight (1 – weight / standard weight) × 100 *Limit of -10	1
CDAI Score			TOTAL

Appendix 2. Patient Reported Outcomes – 2 items (PRO2)

The patient reported outcomes of liquid or very soft stool and abdominal pain (PRO2) score will be assessed as follows:

Number	Variable	Description
1	**Liquid or very soft stool**	**Mean of the daily (liquid or very soft) stool count for 7 days**
2	**Abdominal Pain**	**Mean of 7 days of daily ratings as 0=none, 1=mild, 2=moderate, 3=severe**

35

Each PRO2 score will be rounded to the nearest integer for determination of eligibility and calculation of endpoints.

Appendix 3: SES-CD (Simple endoscopic score for Crohn's disease)

Variables	Score			
	0	1	2	3
Size of ulcers (cm)	None	Aphthous ulcers (0.1 – 0.5)	Large ulcers (0.5 – 2.0)	Very large ulcers (>2.0)
Ulcerated surface (%)	None	<10	10-30	>30
Affected surface (%)	Unaffected segment	<50	50-75	>75
Presence of narrowings	None	Single, can be passed	Multiple, can be passed	Cannot be passed

* Total SES-CD: sum of the values of the 4 variables for the 5 bowel segments. Values are given to each variable and for every examined bowel segment (eg, rectum, left colon, transverse colon, right colon, and ileum)

Appendix 4. CTCAE Grading Scale for Severity of Adverse Events

Please refer to the Common Terminology Criteria for Adverse Events (CTCAE) Version 4.03, which can be found at:

http://evs.nci.nih.gov/ftp1/CTCAE/CTCAE_4.03_2010-06-14_QuickReference_8.5x11.pdf

For AEs associated with laboratory abnormalities, the event should be graded on the basis of the clinical severity in the context of the underlying conditions; this may or may not be in agreement with the grading of the laboratory abnormality.

Appendix 5: IBDQ and SF-36

This questionnaire is designed to find out how you have been feeling during the last two weeks. Please circle only one number for each question.

1. How frequent have your bowel movements been during the last 2 weeks?
   a) Bowel movements as or more frequent than they have ever been
   b) Extremely frequent
   c) Very frequent
   d) Moderate increase in frequency of bowel movements
   e) Some increase in frequency of bowel movements
   f) Slight increase in frequency of bowel movements
   g) Normal, no increase in frequency of bowel movements

2. How often has the feeling of fatigue or being tired and worn out been a problem for you during the last 2 weeks?
   a) All of the time
   b) Most of the time
   c) A good bit of the time
   d) Some of the time
   e) A little of the time
   f) Hardly any of the time
   g) None of the time

3. How often during the last 2 weeks have you felt frustrated, impatient, or restless?
   a) All of the time
   b) Most of the time
   c) A good bit of the time
   d) Some of the time
   e) A little of the time
   f) Hardly any of the time
   g) None of the time

4. How often during the last 2 weeks have you been unable to attend school or work because of your bowel problem?
   a) All of the time
   b) Most of the time
   c) A good bit of the time
   d) Some of the time
   e) A little of the time
   f) Hardly any of the time
   g) None of the time

5. How much time during the last 2 weeks have your bowel movements been loose?
   a) All of the time
   b) Most of the time
   c) A good bit of the time
   d) Some of the time
   e) A little of the time
   f) Hardly any of the time
   g) None of the time

6. How much energy have you had during the last 2 weeks?
   a) No energy at all

b) Very little energy
c) A little energy
d) Some energy
e) A moderate amount of energy
f) A lot of energy
g) Full of energy

7. How often during the last 2 weeks did you feel worried about the possibility of needing surgery because of your bowel problem?
a) All of the time
b) Most of the time
c) A good bit of the time
d) Some of the time
e) A little of the time
f) Hardly any of the time
g) None of the time

8. How often during the last 2 weeks have you had to delay or cancel a social engagement because of your bowel problems?
a) All of the time
b) Most of the time
c) A good bit of the time
d) Some of the time
e) A little of the time
f) Hardly any of the time
g) None of the time

9. How often in the past 2 weeks have you been troubled by cramps in your abdomen?
a) All of the time
b) Most of the time
c) A good bit of the time
d) Some of the time
e) A little of the time
f) Hardly any of the time
g) None of the time

10. How often in the past 2 weeks have you felt generally unwell?
a) All of the time
b) Most of the time
c) A good bit of the time
d) Some of the time
e) A little of the time
f) Hardly any of the time
g) None of the time

11. How often during the last 2 weeks have you been troubled because of fear of not finding a bathroom?
a) All of the time
b) Most of the time
c) A good bit of the time
d) Some of the time
e) A little of the time
f) Hardly any of the time
g) None of the time

12. How much difficulty have you had, as a result of your bowel problems, doing leisure or sports activities you would liked to have done during the last 2 weeks?
a) A great deal of difficulty; activities made impossible

b) A lot of difficulty
c) A fair bit of difficulty
d) Some difficulty
e) A little difficulty
f) Hardly any difficulty
g) No difficulty; no limit sports or leisure activities

13. How often during the last 2 weeks have you been troubled by pain in the abdomen?
a) All of the time
b) Most of the time
c) A good bit of the time
d) Some of the time
e) A little of the time
f) Hardly any of the time
g) None of the time

14. How often during the past 2 weeks have you had problems getting a good night's sleep, or been troubled by waking up during the night?
a) All of the time
b) Most of the time
c) A good bit of the time
d) Some of the time
e) A little of the time
f) Hardly any of the time
g) None of the time

15. How often during the past 2 weeks have you felt depressed or discouraged?
a) All of the time
b) Most of the time
c) A good bit of the time
d) Some of the time
e) A little of the time
f) Hardly any of the time
g) None of the time

16. How often during the past 2 weeks have you had to avoid attending events where there was no bathroom at hand?
a) All of the time
b) Most of the time
c) A good bit of the time
d) Some of the time
e) A little of the time
f) Hardly any of the time
g) None of the time

17. Overall, in the past 2 weeks, how much problem have you had with passing large amounts of gas?
a) A major problem
b) A big problem
c) A significant problem
d) Some trouble
e) A little trouble
f) Hardly any trouble
g) No trouble

18. Overall, in the last 2 weeks, how much of a problem have you had maintaining or getting to the weight you would like to be at?

a) A major problem
b) A big problem
c) A significant problem
d) Some trouble
e) A little trouble
f) Hardly any trouble
g) No trouble

19. Many patients with bowel problems often have worries and anxieties related to their illness. These include worries about getting cancer, worries about never feeling better, and worries about having a relapse. In general, how often during the last 2 weeks have you felt worried or anxious?
a) All of the time
b) Most of the time
c) A good bit of the time
d) Some of the time
e) A little of the time
f) Hardly any of the time
g) None of the time

20. How much of the time during the last 2 weeks have you been troubled by a feeling of abdominal bloating?
a) All of the time
b) Most of the time
c) A good bit of the time
d) Some of the time
e) A little of the time
f) Hardly any of the time
g) None of the time

21. How often during the last 2 weeks have you felt relaxed and free of tension?
a) None of the time
b) A little of the time
c) Some of the time
d) A good bit of the time
e) Most of the time
f) Almost all of the time
g) All of the time

22. How much time during the last 2 weeks have you had a problem with rectal bleeding with your bowel movements?
a) All of the time
b) Most of the time
c) A good bit of the time
d) Some of the time
e) A little of the time
f) Hardly any of the time
g) None of the time

23. How much time during the last 2 weeks have you felt embarrassed as the result of soiling, or because of an unpleasant odor caused by your bowel movement?
a) All of the time
b) Most of the time
c) A good bit of the time
d) Some of the time
e) A little of the time
f) Hardly any of the time

g) None of the time

24. How much of the time during the past 2 weeks have you been troubled by a feeling of having to go to the bathroom even though your bowels are empty?
a) All of the time
b) Most of the time
c) A good bit of the time
d) Some of the time
e) A little of the time
f) Hardly any of the time
g) None of the time

25. How much of the time during the last 2 weeks have you felt tearful of upset?
a) All of the time
b) Most of the time
c) A good bit of the time
d) Some of the time
e) A little of the time
f) Hardly any of the time
g) None of the time

26. How much of the time during the last 2 weeks have you been troubled by accidental soiling of your underpants?
a) All of the time
b) Most of the time
c) A good bit of the time
d) Some of the time
e) A little of the time
f) Hardly any of the time
g) None of the time

27. How much of the time in the 2 weeks have you felt angry as a result of your bowel problems?
a) All of the time
b) Most of the time
c) A good bit of the time
d) Some of the time
e) A little of the time
f) Hardly any of the time
g) None of the time

28. To what extent has your bowel problem limited sexual activity during the last 2 weeks?
a) No sex as a result of Crohn's disease
b) Major limitation as a result of Crohn's disease
c) Moderate limitation as a result of Crohn's disease
d) Some limitation as a result of Crohn's disease
e) A little limitation as a result of Crohn's disease
f) Hardly any limitation as a result of Crohn's disease
g) No limitation as a result of Crohn's disease

29. How much of the time during the last 2 weeks have you been troubled by feeling sick to your stomach?
a) All of the time
b) Most of the time
c) A good bit of the time
d) Some of the time
e) A little of the time
f) Hardly any of the time
g) None of the time

30. How much of the time during the past 2 weeks have you felt irritable?
    a) All of the time
    b) Most of the time
    c) A good bit of the time
    d) Some of the time
    e) A little of the time
    f) Hardly any of the time
    g) None of the time

31. How often during the last 2 weeks have you felt a lack of understanding from others?
    a) All of the time
    b) Most of the time
    c) A good bit of the time
    d) Some of the time
    e) A little of the time
    f) Hardly any of the time
    g) None of the time

32. How satisfied, happy, or pleased have you been with your personal life during the past 2 weeks?
    a) Very dissatisfied, unhappy most of the time
    b) Generally dissatisfied, unhappy
    c) Somewhat dissatisfied, unhappy
    d) Generally satisfied, pleased
    e) Satisfied most of the time, happy
    f) Very satisfied most of the time, happy
    g) Extremely satisfied, could not have been more happy or pleased

SF36 Health Survey

INSTRUCTIONS: This set of questions asks for your views about your health. This information will help keep track of how you feel and how well you are able to do your usual activities. Answer every question by marking the answer as indicated. If you are unsure about how to answer a question please give the best answer you can.

1. In general, would you say your health is: (Please tick **one** box.)
   - Excellent ☐
   - Very Good ☐
   - Good ☐
   - Fair ☐
   - Poor ☐

2. Compared to one year ago, how would you rate your health in general now? (Please tick **one** box.)
   - Much better than one year ago ☐
   - Somewhat better now than one year ago ☐
   - About the same as one year ago ☐
   - Somewhat worse now than one year ago ☐
   - Much worse now than one year ago ☐

3. The following questions are about activities you might do during a typical day. Does your health now limit you in these activities? If so, how much? **(Please circle one number on each line.)**

	Activities	Yes, Limited A Lot	Yes, Limited A Little	Not Limited At All
3(a)	**Vigorous activities**, such as running, lifting heavy objects, participating in strenuous sports	1	2	3
3(b)	**Moderate activities**, such as moving a table, pushing a vacuum cleaner, bowling, or playing golf	1	2	3
3(c)	Lifting or carrying groceries	1	2	3
3(d)	Climbing **several** flights of stairs	1	2	3
3(e)	Climbing **one** flight of stairs	1	2	3
3(f)	Bending, kneeling, or stooping	1	2	3
3(g)	Waling **more than a mile**	1	2	3
3(h)	Walking **several blocks**	1	2	3
3(i)	Walking **one block**	1	2	3
3(j)	Bathing or dressing yourself	1	2	3

4. During the past 4 weeks, have you had any of the following problems with your work or other regular daily activities as a result of your physical health?

	(Please circle one number on each line.)	Yes	No
4(a)	Cut down on the **amount of time** you spent on work or other activities	1	2
4(b)	Accomplished less than you would like	1	2
4(c)	Were **limited** in the **kind** of work or other activities	1	2
4(d)	Had **difficulty** performing the work or other activities (for example, it took extra effort)	1	2

5. During the past 4 weeks, have you had any of the following problems with your work or other regular daily activities as a result of any emotional problems (e.g. feeling depressed or anxious)?

	(Please circle one number on each line.)	Yes	No
5(a)	Cut down on the **amount of time** you spent on work or other activities	1	2
5(b)	Accomplished less than you would like	1	2
5(c)	Didn't do work or other activities as **carefully** as usual	1	2

6. During the past 4 weeks, to what extent has your physical health or emotional problems interfered with your normal social activities with family, friends, neighbours, or groups? (Please tick **one** box.)

- Not at all ☐
- Slightly ☐
- Moderately ☐
- Quite a bit ☐
- Extremely ☐

7. How much physical pain have you had during the past 4 weeks? (Please tick **one** box.)

- None ☐
- Very mild ☐
- Mild ☐
- Moderate ☐
- Severe ☐
- Very Severe ☐

8. During the past 4 weeks, how much did pain interfere with your normal work (including both work outside the home and housework)? (Please tick **one** box.)

- Not at all ☐
- A little bit ☐
- Moderately ☐
- Quite a bit ☐
- Extremely ☐

9. These questions are about how you feel and how things have been with you during the past 4 weeks. Please give the one answer that is closest to the way you have been feeling for each item.

	(Please circle one number on each line.)	All of the Time	Most of the Time	A Good Bit of the Time	Some of the Time	A Little of the Time	None of the Time
9(a)	Did you feel full of life?	1	2	3	4	5	6
9(b)	Have you been a very nervous person?	1	2	3	4	5	6
9(c)	Have you felt so down in the dumps that nothing could cheer you up?	1	2	3	4	5	6
9(d)	Have you felt calm and peaceful?	1	2	3	4	5	6
9(e)	Did you have a lot of energy?	1	2	3	4	5	6
9(f)	Have you felt downhearted and blue?	1	2	3	4	5	6
9(g)	Did you feel worn out?	1	2	3	4	5	6
9(h)	Have you been a happy person?	1	2	3	4	5	6
9(i)	Did you feel tired?	1	2	3	4	5	6

10. During the past 4 weeks, how much of the time has your physical health or emotional problems interfered with your social activities (like visiting with friends, relatives etc.) (Please tick **one** box.)

- All of the time ☐
- Most of the time ☐
- Some of the time ☐
- A little of the time ☐
- None of the time ☐

11. How TRUE or FALSE is each of the following statements for you?

	(Please circle one number on each line.)	Definitely True	Mostly True	Don't Know	Mostly False	Definitely False
11(a)	I seem to get sick a little easier than other people	1	2	3	4	5
11(b)	I am as healthy as anybody I know	1	2	3	4	5
11(c)	I expect my health to get worse	1	2	3	4	5
11(d)	My health is excellent	1	2	3	4	5

Thank You!

Appendix 6: Routine Study Monitoring Plan provided by UCLA CTSI Office of Regulatory Affairs' (ORA) Quality Assurance Team.

The CTSI DSMB meets monthly to review all SAE reports for trials overseen by the CTSI DSMB. All SAE reports, which have been filed since the previous meeting, are presented to the Board for review. SAEs shall be reported to the CTSI DSMB in a timely manner [ten days, two days for a death] regardless of relationship and expectedness. The CTSI DSMB SAE reporting algorithms are outlined in Appendix B. The CTSI ORA will review all submissions and the ORA staff will enter the information into the OnCore database. Reports are generated for full CTSI DSMB review. For trials where the CTSI DSMB has primary DSMB review responsibility, the DSMB requires that the PI generate cumulative adverse event reports for quarterly, biannual or annual review, as determined by the DSMB.

The DSMB reviews each SAE report and determines whether or not protocol modifications are warranted to ensure subject safety. In this review, prior occurrences of similar toxicity with the therapy under study are taken into consideration, as well as the severity of the event and the likelihood that it was related to a study drug. The DSMB may recommend no changes to the study if the event is expected or related to other causes such as the subject's underlying condition. The DSMB may request an un-conflicted expert's advice with national experience to support their deliberations and decisions.

The CTSI DSMB reviews all dose-limiting toxicities (DLTs) for all dose-escalation studies. Protocol suspensions and re-opening of accrual to the next cohort based on DLT evaluation fall under the purview of the DSMB. For phase II and III studies, early stopping rules and interim analyses are reviewed for appropriateness as well. For Phase III studies, both toxicity and efficacy monitoring is required.

The CTSI DSMB has the authority to immediately halt a study (i.e., discontinuation of any further treatment of enrolled subjects and discontinuation of enrollment of new subjects) should there be any serious unexpected toxicity that warrants further investigation. The Principal Investigator is responsible for reporting to the appropriate agencies, including the FDA, if they are suspended from conducting research. The CTSI ORA staff will work with the Investigator to ensure that the proper reporting procedures are followed.

Requests for single subject exceptions/waivers from the approved study protocol, including out of window procedures and eligibility deviations, must be reviewed and approved by the DSMB. A physician member of the Board will review each exception/waiver request. Approvals and disapprovals of the request are sent to the Principal Investigator via email and copied to the UCLA IRB. Requests for single subject exceptions/waivers are made by the Principal Investigator via email utilizing the "Single Subject Exception Request Form." This form is located in Appendix D.

CTSI DSMB correspondences are addressed to the Principal Investigator and copied to the UCLA IRB. Minutes of the DSMB meetings are maintained in a computer file.

Confidentiality: Each member of the CTSI DSMB is responsible for maintaining strict confidentiality of the study data. Members will not share any study data or information about the study with any individual external to the DSMB. Communication of deliberations or

recommendations of the DSMB, either written or oral, should not be made outside of the Board. Outcome results are strictly confidential and must not be divulged to any non-member of the DSMB except in those cases where DSMB is required to inform the UCLA IRB of its determinations. Disclosure of outcome results to the IRB must only occur with written approval of the DSMB. The DSMB reviews unblinded data. All unblinded data will be maintained in a confidential manner and not shared with anyone outside of the DSMB. A member who believes he or she may have a potential intellectual or financial conflict of interest during the course of review of the data must inform the chairperson of the DSMB. In such case, the meeting minutes will record the disclosure of the potential conflict of interest and that the individual recuse himself from the discussions and abstains from voting on the DSMB decision.

Level of Risk of a Study

The CTSI DSMB will determine the degree of risk of the study and will ensure that there are procedures in place to ensure the safety of the subjects that are enrolled in the trial. The intensity level of study oversight is determined by the risk category. Some of the factors that are considered when assigning the Level of Risk category include:

- A biostatistical design and appropriate procedures for proper data management so that the information collected can be properly validated.
- Appropriate Serious Adverse Event reporting procedures must be in place.
- The study duration must be appropriate and must be based on a realistic rate of enrollment.
- Data collection and data management must be adequate to verify and ensure subject eligibility.

Assignment of risk

Assigning risk ensures that the data and safety monitoring is based on the level of risk (low, medium, or high) to ensure that the data and safety monitoring activities are appropriate. Below are some of the criteria used to make a decision regarding the assignment of risk:

- Expected duration of the study based upon the estimated rate of enrollment.
- Type of study population (e.g., children, geriatric).
- The procedures used in the trial are commensurate with the degree of risk.
- Adequate data management systems in place and appropriate case report forms.
- Proper serious adverse event reporting procedures in place.
- Proper biostatistical design and data analysis procedures in place.

Level 2

Levels of Risk

Level 1

Example of type of trial: Investigator held IND of high complexity or of high toxicity potential. Examples are dendritic cell products from GMP suite, or an experimental agent still in phase I/II development. Typically, these clinical trials involve the first use of the drugs in humans, so only the investigator is knowledgeable about the test article.

- The Compliance Officer meets with PI/Staff prior to study initiation and reviews regulatory requirements and operating system. Compliance Officer provides real time monitoring to determine eligibility prior to enrollment onto the protocol.
- Real time QA monitoring of the subjects and data collection occurs for all subjects entered onto the trial.
- Comprehensive QA auditing within first year or first 10 subjects enrolled, whichever comes first. Subsequent audit frequency will be annually.
- Frequency of DSMB Summary Report is typically on a quarterly basis.

Level 2

Example of type of trial: Institutional study for which IND is exempt by FDA or has an IND, but the drug is approved by the FDA for a different indication and is in Phase II or III. Examples are studies using commercially available agents for an unapproved indication based on standard regimen.

- Compliance Officer meets with PI/Staff prior to study initiation; review regulatory requirements and operating system. Compliance Officer provides real time monitoring to determine eligibility prior to enrollment onto the protocol.
- Real time QA monitoring of the subjects and data collection occurs for all subjects entered onto the trial.
- Comprehensive QA auditing within first year or first 10 subjects enrolled, whichever comes first. Subsequent audit frequency will be annually.
- Frequency of DSMB Summary Report is typically on a biannual basis or approximately every six months.

Level 3

Example of type of trial: Institutional trials with low risk and not monitored by external systems (e.g., dietary intervention trials).

- Compliance Officer meets with PI/Staff prior to study initiation; review regulatory requirements and operating system.
- QA comprehensive audits are conducted every 18 months.
- Frequency of DSMB Summary Report is typically on an annual basis or approximately every 12 months.

MONITORING/AUDITING ACTIVITIES:

The Compliance Officer of the CTSI Office of Regulatory Affairs (ORA) will monitor and audit the clinical records for all human subjects enrolled onto trials overseen by the CTSI DSMB. The CTSI ORA Compliance Officer will perform real time review of informed consent processes and

the meeting of all inclusion and exclusion criteria and screening results at study entry. Active monitoring will offer the study teams with prospective information that can be used to enhance the quality of research being performed contemporaneously. Auditing is a review of historic performance of the research effort and is performed on case report forms, regulatory files and source documents to measure the quality of the research effort in a retrospective manner.

Detailed Reporting Mechanism for Adverse Events

An adverse event is any undesirable experience associated with the use of a medical product in a subject (any unfavorable and unintended sign, symptom or disease temporally associated with the use of a medical treatment or procedure regardless of whether it is considered related to the medical treatment or procedure). Any clinical adverse event must be recorded on the case report form during the course of the study. The investigator must evaluate and document the adverse event for severity, and grade it.

CTCAE version 5 should be used in the grading and selection of SAE terminology.

https://ctep.cancer.gov/protocoldevelopment/electronic_applications/docs/CTCAE_v5_Quick_Reference_5x7.pdf

If the event is Serious, it should be reported immediately (in writing within 10 days of awareness, 48 hours for death) by the Principal Investigator to the IND sponsor, CTSI DSMB (only if the trial is overseen by the CTSI DSMB) and to any appropriate agency (according to the agency's requirements); UCLA IRB; the NIH and the UCLA IBC for gene medicine trial; and directly to the FDA if it is an institutional IND trial. For institutional trials, the CTSI ORA Compliance Officer will assist the investigator to ensure that all serious adverse events are properly documented and reported in accordance to federal and institutional requirements.

All serious adverse events that occur in a research study overseen by the CTSI DSMB must be submitted to the CTSI DSMB, regardless of relationship, expectedness, or severity. Those adverse events that are not considered serious should be reported to the CTSI DSMB in the next DSMB Summary Report submission.