A randomized control study to examine the influence of a healthy diet on moderate to severe ulcerative colitis patients undergoing tofacitinib induction

National Clinical Trial (NCT) identified Number: NCT04505410

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Sponsor: University of Miami Miller School of Medicine

Funded by: Pfizer (PI-initiated Study)

Version Number: 3, 10/05/2021

IRB Study Number: 20200436

1) Protocol Title

A randomized control study to examine the influence of a healthy diet on moderate to severe ulcerative colitis patients undergoing tofacitinib induction

2) Objectives*

The overarching goal is to use fasting mimicking diet (FMD) as adjunctive treatment for UC patients undergoing induction with biological medications (tofacitinib, anti-TNFs, ustekinumab or vedolizumab), and to identify microbiome and metabolome changes occurring as a result of the diet + biologic medication intervention. This will provide the foundation for predicting response to therapy. The objectives of this study are 1) to determine if a FMD dietary intervention improves clinical response to biologic medication 8-week induction therapy in UC 2) to identify predictors of clinical response to biologic medications in the baseline dietary pattern and 3) to identify changes in the stool microbiome and metabolome that could predict response. The study population will include adult participants with moderate to severe ulcerative colitis who are starting tofacitinib or other biologic medications at our University of Miami Crohn's and Colitis Center.

3) Background*

Ulcerative colitis (UC) is a chronic disease that can lead to devastating consequences including colectomy [1]. Medical therapy has improved significantly but there are still many patients with poor response to therapy [1]. For example, in OCTAVE induction trials 1 and 2, approximately 59.9% of patients had clinical response to tofacitinib but this still left 40.1% who did not show any improvement by week 8 [2]. With that as a backdrop, diet offers a safe opportunity to complement immune-based therapy in sick UC patients. The effect of diet may be only on symptoms but may also be on inflammation. To date, there is only one published study that has looked at the impact of diet on reducing symptoms of an active UC flare-up [3]. This small study was focused on reducing symptoms not inflammation [4]. Additionally, there are no studies in UC that have proposed to combine diet and medical therapy to improve clinical response. A study in Crohn's disease looked at a plant-based diet as adjunctive therapy to infliximab and found that 96% achieved clinical remission by week 6 compared to published remission rates of 64% using monotherapy alone [5,6]. This study demonstrates the potential of using diet as a safe adjunctive therapy to biologics and lays the foundation for this proposal. We propose to evaluate the effect of the fasting mimicking diet (FMD) on clinical response. We will use this diet because of its reported efficacy in reducing colonic inflammation, improving intestinal regeneration, and expansion of beneficial bacteria like *Lactobacillacea and Bifidobacteriacea.*[12]

A great deal of research has been devoted to identifying genetic and microbial factors underpinning IBD. In spite of the investments made in this type of research, we still have not been able to develop actionable strategies based on this knowledge. In particular, diet has the possibility of changing the microbiome and metabolome to reduce the signs and symptoms of UC. Our group has recently completed a small but comprehensive study in UC patients in remission and found that a low fat, high fiber diet can improve quality of life and lower serum levels of inflammatory markers. But no one has specifically performed a prospective, controlled study of diet in the context of a sick UC patient and paired with an effective drug. Moreover, little is known about the effects of dietary + drug manipulation on the human microbiota and metabolome. *Therefore, this study seeks to determine whether intervals of FMD will enhance response to biologic therapy by week 8 in a randomized, diet placebo-controlled*

trial. We will measure baseline diet patterns and evaluate whether a change in diet influences clinical response. By measuring the baseline microbiome in the stool and again after the last FMD interval, we will be able to measure changes occurring as a result of diet intervention. We will use tofacitinib and available FDA approved biologic therapies available for the treatment of UC (anti-TNFs, vedolizumab and ustekinumab) to examine the impact of diet on UC clinical response. Embedded within this proposal are the seeds of future studies that will test dietary interventions incorporating predictive factors we will identify in this proposal.

Innovation. Our project is novel for the followed reasons:

- A proper, randomized controlled study of a protocolized dietary intervention in active ulcerative colitis has not been done. This will potentially eliminate the confounders introduced by uncontrolled, under-powered, diet study designs available.
- Our study will include patients who have active moderate to severe UC since these types of patients are typically excluded from dietary studies but are actually the ones that need the most help.
- The fasting mimicking diet (FMD) is a novel dietary approach. Animal and human studies demonstrate efficacy in reducing colonic inflammation.
- Microbiome or metabolome changes have not been studied in active UC using diet as treatment. These microbiome changes may identify mechanisms by which diet may be helpful.

This study builds upon prior existing work done including diet studies performed at our institution. Below we describe existing evidence and our own pilot data that explain the background for this proposal.

Preliminary data

Existing Evidence

Diet can influence ongoing inflammation in UC. There are multiple studies demonstrating that a "western diet" characterized by a diet high in animal protein, processed foods, and low in fiber from fruits and vegetables, is associated with a greater risk of developing inflammatory bowel disease (IBD).[8, 9,10] Recent studies suggest that a diet that in turn "prudent diets", low in Western dietary components, may in turn help reduce colonic inflammation.[8] Beyond epidemiologic data, we have a greater understanding of the role that diet may play in prevention of relapse in IBD. However, most studies however have focused on diets that may help in Crohn's disease but not in UC.[9] There is also a lack of randomized controlled studies with protocolized diets that will measure the effects of diet on inflammation and who seek to understand mechanisms of response. Most studies of diet in UC are biased by small sample sizes, uncontrolled methods and there are no well designed studies aiming to decrease active inflammation in UC.

Fasting Mimicking Diet (FMD) is a novel diet that may help attenuate inflammation in UC. The fasting mimicking diet, is a novel low calorie diet that is characterized by episodes of intermittent "fake" fasting, that has the potential to work in attenuating inflammation in UC.[12] Prior studies demonstrate that calorie restriction can improve aging, dementia, metabolic syndrome, obesity and diabetes. [11] Intermittent fasting elicits an adaptive cellular response that improves glucose regulation, increases stress resistance, and suppresses inflammation [11]. During intermittent fasting there is activation of cellular pathways that enhance intrinsic defenses against oxidative and metabolic stress

and those that remove and repair damaged molecules [11]. Pertinent to UC, cells respond to intermittent fasting by engaging in a coordinated adaptive stress response that leads to increased expression of antioxidant defenses, DNA repair, and autophagy, and down- regulation of inflammation. There is a clear association between a dysregulated autophagy pathway and development of IBD, providing a mechanism by which this diet may help in IBD. Further, following a fasting period, cells then engage in tissue specific processes of growth and plasticity that can bolster healing [12].

The FMD allows participants to consume meals and snacks while at the same time maintaining the properties that mimic fasting and elicit similar cellular responses. Prior studies using the FMD have examined 5 days of "fasting" with intervals of 25 days in between each fasting[12]. The diet is low in carbs and protein yet high in healthy fats such as olives and flax. fats. All meals and snacks are whole-food derived and plant based. Day one of the diet provides ~1,090 kcal (10% protein, 56% fat, 34% carbs), while days two through five provide only 725 kcal (9% protein, 44% fat, 47% carbs), designed to provide ~54% of normal caloric intake. A recently published study examined the effects of this fasting diet in animal models of colitis and in an uncontrolled study of 26 humans[12]. In murine models of colitis, intervals of the FMD reduced intestinal inflammation and CRP, promoted intestinal regeneration and stimulated growth of protective gut populations such as *Lactobacillacea* and *Bidifidobacteriacea.*[12] In the human sample of UC patients, FMD was safe, feasible and effective in reducing systemic inflammation (measured as total white blood cell count and lymphocytes).[12] *These studies highlight a sensible mechanism by which intermittent fasting may help reduce colitis including via resetting of the autophagy pathways. The FMD is an improvement of the intermittent (water) fasting diet that may help inflammation while limiting weight loss and improving patient tolerability.*

The microbiome and diet-induced metabolites are a critical link between diet and intestinal inflammation in UC and may permit the development of biomarkers of response. Diet is a major driver of the intestinal microbiome [13,14] and downstream metabolites and as mentioned it also plays a critical role in the pathogenesis of UC [13-16]. Multiple studies have shown that there is a dramatic decrease in bacterial diversity in patients with UC, especially in bacteria known to produce short chain fatty acids (SCFA)[17- 20], which have anti-inflammatory properties [17,21,22]. When healthy humans were placed on an animal-based diet, rich in fat and protein, there was a rapid decrease in abundance of SCFA-generating bacteria like *Roseburia* [16] and an increase in *Bacteroides* [23], whichcan induce colitis in genetically susceptible mice [24]. Animal based diets are also associated with increases in Enterobacteriaceae including Escherichia, associated with inflammation in IBD patients [25]. Changes in the UC gut microbiome are associated with changes in the gut metabolome [26]. These data suggest that the microbiome/metabolome transduces many aspects of diet as treatment for UC. It also suggests that specific microbial/metabolic signatures may be useful to predict responses to specific diets.

Preliminary data from our Crohn's and Colitis Center:

A low fat diet (LFD) improves QoL and systemic inflammation in patients with UC. We have performed a pilot study of a LFD versus HFD intervention in UC patients that were in remission to mildly active. The study was a randomized, parallel-group, cross- over study of two 4-week periods with each participant serving as her/his own control. We have completed 18 UC patients who received a catered, LFD or a HFD. We targeted patients with inactive or only mildly active disease. The HFD arm was based on NHANES data where 35-40% of the calories are from fat with a 20-30:1 ratio of omega-6/omega-3 fatty acids with less than 12 g of fiber per day. In the LFD arm, 10% of calories are from fat, with an approximate ratio of 1:1 of omega-6/omega-3 fatty acids with 25-35 g fiber per

day which is the USDA recommended amount for women and men. The adherence to the diet intervention using the patients self-reported data as entered in Nutrihand is quite high and matches the preset goals. In our preliminary data, we have demonstrated a significant improvement in quality of life (short IBD questionnaire) after a low fat/high fiber diet (p=0.03), and lowered serum amyloid levels, a marker of mucosal healing. *These data justify using a low saturated fat diet such as the Fasting Mimicking Diet in UC to decrease clinical symptoms, improve QoL, and decrease inflammation.*

A controlled diet intervention with a low fat diet (LFD) in UC leads to changes in the microbiome and metabolome. Our laboratory is well-versed in studies involving the intestinal microbiome/metabolome in humans and animal models. Preliminary analysis of the microbiome in 18 patients who completed a pilot diet study demonstrate there is no statistical significant change at beta/alpha diversity in the microbiome after the LFD. However, we found a significant difference at the phylum level. In LFD, Actinobacteria (p=0.02) decreased while Bacteroidetes (p=0.05) increased (Figure 3). Furthermore, at the genus level there was a decrease in Bifidobacterium (p=0.04); Collinsella (p=0.03); Coprococcus (p=0.05) and an increase in Prevotella (p=0.008) and Blautia (p=0.016) (data not shown). We also found significant microbiome changes at Beta diversity between patients that started a LFD compared to patients that started first on HFD (data not shown). For the metabolome, we used a targeted and an untargeted analysis of stool. We have validated our preliminary untargeted metabolomics of 44 fecal samples (11 patients at 4 time points) data with PLS-DA (Partial Least Squares discriminant analysis) model to determine the metabolomic differences between the diets. Our preliminary data revealed striking changes of metabolites between Baseline and LFD (Figure 4). Additionally, based on VIP score (>1), we have identified a few putative compounds from the untargeted approach at baseline (i.e. Lauric acid and Dopaquinone) which are normally high in UC and CD[27]. After LFD, these 2 metabolites were decreased but omega- 3 fatty acids were increased, which is known to reduce inflammation[27]. *Together these data not only demonstrate that we are capable of performing microbiome/metabolomics experiments, but it also provides justification to pursue the identification and role of dietary metabolites in response to a diet low in saturated fats, such as the FMD.*

4) Inclusion and Exclusion Criteria*

Inclusion: We will include adult patients with a confirmed diagnosis of UC living in South Florida, a population that is ethnically diverse. Patients to be included will have moderate to severely acting UC, defined as active disease on the SCCAI (>2), with presence of rectal bleeding

Exclusions: The presence of clinical findings suggestive of Crohn's disease, clinical signs of fulminant colitis, toxic megacolon, or indeterminate, microscopic, ischemic, or infectious colitis or impending hospitalization for severe ulcerative colitis.. Other exclusion criteria include: patients with allergies to nuts/soy/sesame/oats/celery or celeriac, patients that are diabetic on a glucose lowering drug, individuals with a history of syncope/presyncope with fasting or from medical conditions, women who are pregnant or nursing, individuals with very low BMI<18 and patients, and patients with the following comorbidities: chronic kidney disease, diabetes, active cancer. Permitted concomitant medications for ulcerative colitis will be oral aminosalicylates and oral glucocorticoids. Given the study population includes a sick cohort with active symptoms, we will need to permit the use of oral corticosteroids as many patients biologic medications will be on them. Because of the anticipated variability in the consumption of oral steroids, we will take prednisone dose into account

in our multivariable logistic regression model (using dose as a continuous covariate which will allow us to factor in differences in dosage). Prohibited concomitant therapies will include azathioprine, methotrexate, and mercaptopurine. Additionally, patients with a history of routinely fasting, patients who do not like the food items that form part of the kits for the fasting mimicking diet or patients with dietary restrictions. Patients with a fever, cough, respiratory distress or other symptoms of an active infection. Patients who are adults but unable to consent, minors, pregnant women and prisoners are excluded from participation.

5) Number of Subjects*

In this study we will include a total of 76 patients (38 per arm). This medium-sized study will be essential to lay the groundwork to add diet into the IBD treatment algorithm.

6) Study-Wide Recruitment Methods*

This study will recruit patients from the University of Miami Center for Crohn's and Colitis and from Gastro Health, a large medical, private-practice group located in Kendall, Florida.

Gastro Health is made up of board-certified physicians and allied health professionals specializing in the treatment of gastrointestinal disorders, nutrition and digestive health. Gastro Health physicians are involved in clinical research and medical education and currently collaborate with the Division of Gastroenterology on multiple research studies involving IBD. Thus, adding this FMD study would be an ideal and seamless process.

7) Recruitment Methods

The University of Miami Center for Crohn's and Colitis serves 5,000 patients with IBD from which to recruit ethnically-diverse study participants. Our prior experience with conducting diet studies is evidenced in the preliminary data. Participants will be recruited during routine primary care visits, using physician-centered recruitment at the Crohn's and Colitis Center at the University of Miami and at Gastro Health. To promote interest in physician-centered recruitment, we will educate the physicians at the University of Miami Center for Crohn's and Colitis and at Gastro Health about the study's aims and procedures prior to the study's start.

We will ensure that information will be available to facilitate physician referrals. Physicians from both sites will be able to initiate a referral for any interested patient who they feel may benefit from involvement. We are also going to use flyers so that patients may contact us.

For their participation in this study participants will be compensated a total of $60 in monetary gift cards. The participant may choose to receive a $30 gift card after completing visit 1, this includes initial baseline questionnaires, blood draw and stool kit and a second $30 gift card after completing Visit 2 (week 7), this includes food diaries, follow-up questionnaires, and a second set of blood draw and stool kit. They may also choose to receive the full $60 altogether after completing both study visits. If a participant withdraws from the study, they may only be compensated for any study visit completed.

Additionally, if participants are starting tofacitnib therapy participants will receive the tofacitinib pills for a total of 8 weeks. This will not be processed through their insurance and will not require

authorization. However, participants will be advised that authorization of their insurance will be required for tofacitinib after 8 weeks. If participants are starting any other biologic medication therapy this will be processed through their insurance.

Valet parking tickets will also be validated each time the subject visits the clinic for study-related visits.

8) **Study Timelines***

Seventy-six patients will be enrolled at different time points throughout this study in groups of approximately ten. Each patient will be an active participant in the study for a total of 8 weeks. A complete breakdown of the study timeline is provided below:

Milestones and Timeline (1-3 months)
IRB: Protocol/Consent review. IRB approval within 1-2 months. *We had a similar pilot diet study we do not anticipate delay
Order fasting mimicking diet kits for the first set up of patients --1 month to receive
Creation of RedCap database to manage clinical data and biospecimens
Set up randomization
Research coordinator will be trained by dietician on diet methods for initial recruitment and follow up.
Milestones (3-6 months)
Enroll 10 participants using a 1:1 randomization strategy
Follow up visits of enrolled participants
Inflammatory markers preparation of collected samples
Genomic DNA preparation of collected samples
Milestones (6-12 months)
Re-evaluation of study methods, compliance of fasting intervals and adjustments if needed.
Reorder fasting mimicking kits for next set of patients
Enroll next 10 participants using a 1:1 randomization strategy
Follow up visits of enrolled participants
Inflammatory markers preparation of collected samples

genomic DNA preparation of collected samples
Preliminary analyses and evaluation of results for possible presentation at national conferences
Milestones (12-15 months)
Enroll next 20 participants using a 1:1 randomization
Follow up visits of enrolled participants
Inflammatory markers preparation of collected samples
Genomic DNA preparation of collected samples
Milestones (16 months-24 months)
Reordering of FMD kits
Enroll next 20 participants using a 1:1 randomization
Follow up visits of enrolled participants
Inflammatory markers preparation of collected samples
genomic DNA preparation of collected samples
Milestones (2 years-2.5 yrs)
Enroll last 18 participants using a 1:1 randomization
Follow up visits of enrolled participants
Inflammatory markers preparation of collected samples
genomic DNA preparation of collected samples
Begin to gather preliminary analyses, meet with biostatisticians, submit abstracts to large national conferences.
Milestones (2.6 yrs-3 years)
Shipment of samples
Biostatistical analysis
Interpretation of results and manuscript preparation (planned submission to Gastroenterology journal).

9) Study Endpoints*

On the premise of the existing literature and our own preliminary data, we propose a fasting mimicking diet as a non-pharmacological treatment for patients with active moderate to severe UC to augment clinical response to biologic medication induction therapy. Our application further aims to identify baseline dietary patterns and microbiome factors that predict enhanced response to a fasting mimicking diet as adjunctive therapy to biologic medication induction.

Aim 1. To determine whether a fasting mimicking diet used as adjunctive therapy during biologic induction enhances response to therapy in patients with moderate to severe UC.
Questions to be answered:

1. *Does consumption of two 5-day intervals of a FMD improve clinical response to tofacitinib or other biologic medications (Anti-TNFs, vedolizumab, ustekinumab)by week 8 compared to medications alone?*
2. *Does performing two 5-day intervals of a FMD improve biochemical markers of inflammation by week 8 compared to tofacitinib or other biologic medications alone?*

Rationale: We poorly understand which diet is most effective at attenuating colonic inflammation in UC. At present, patients who are actively flaring are advised to adhere to a low fiber diet to minimize symptoms; but this diet has no role in reducing inflammation and is associated with microbial dysbiosis in the long term[28]. Prior studies show that the FMD decreases colonic inflammation, stimulates intestinal regeneration, and promotes growth of healthy intestinal bacteria like *Lactobacilli* that can in turn regulate T cell activity and reduce the severity of IBD symptoms [29]. *We therefore hypothesize that patients who adhere to two cycles of a FMD within the 8 week induction period of biologic medications are more likely to have a clinical response than patients who follow a standard (low fiber diet) and biologic medicationtherapy by week 8.*

Health Outcomes: The primary end-point will be clinical response at 8 weeks. Clinical response will be assessed using the simple clinical colitis activity index (SCCAI), a validated measure of disease activity in UC (see ref). The SCCAI questionnaire will be completed by the physician, based on interview and examination of the patient. Variables assessed include bowel frequency (day and night), urgency, blood in stool, general well- being and presence of extra-intestinal manifestations. A higher SCCAI indicates greater disease activity (range of 0-19), and a clinical response is defined as a SCCAI decrease of ≥2 points from baseline. Secondary outcomes will be: clinical remission, defined as a SCCAI of ≤ 2 by week 8, steroid-free clinical response and remission, and improvement of biochemical markers including CRP and stool fecal calprotectin.

Co-variates collected: We will collect baseline body weight, age, and disease severity and again at 8 weeks. At baseline we will collect gender, ethnicity and race, as well as a prior history of their disease (location of disease and disease severity included). Any co- variate determined to influence disease activity or baseline diet will be accounted for in multivariable regression models.

Aim 2. To determine whether baseline dietary patterns influence clinical response to a fasting mimicking diet intervention.

Questions to be answered:

A. Does a baseline dietary pattern high in Western dietary components predict

response to FMD intervention?

B. Are there non-Western diet patterns that predict response FMD intervention?

Rationale: There are no studies examining whether baseline diet influences response to therapy in IBD. The diet of patients with UC varies widely based on diet tolerance and often has poor nutritional value, including higher consumption of refined carbohydrates that are characteristic of Western dietary patterns [35]. Prior studies show that diet patterns capture a more sensitive global assessment of diet than individual food groups or macro/micronutrients alone [35]. A Western dietary pattern is a diet high in processed foods, animal protein, and low in fruits/vegetables previously associated with IBD onset (ref). *We hypothesize that those who score high in a Western dietary pattern score and then undergo FMD intervals will likely have the most clinical response compared to those who score lower in the Western pattern score and undergo a FMD intervention.*

Expected Outcomes/Results: We anticipate that a baseline diet pattern that is consistent with a high Western dietary score will likely result in more significant improvement in clinical symptoms than those whose baseline diet pattern is lower in the Western diet score. In other words, an "unhealthier" diet at baseline, will result in the most improvement of symptoms after a change in diet to the FMD intervention. This is under the premise that if patients score low in a Western diet pattern, they are more likely to consume healthy foods, and therefore less likely to benefit from a diet intervention study.

Aim 3. To dissect the effects of a fasting mimicking diet intervention on the microbiome and the metabolome in UC.

Questions to be answered:

A. What is the effect of a FMD on the fecal microbiome composition and metabolome?

B. What are possible predictors of response to tofacitinib or other biologic medication therapy plus FMD in the microbiome composition?

Aim 3A- Examine microbiome and metabolome changes in UC patients in response to FMD plus biologic medication vsbiologic medication . Diet may improve clinical response to biologic therapies by changing the microbiome. Previous studies have not adequately evaluated the impact of a protocolized diet on active UC inflammation. We know that dietary modification can rapidly change the human gut microbiome, making it likely that diet-based approaches can sustain a healthy microbiota which may be a more beneficial approach to long term UC management[39,40,41]. Human studies have not addressed the effects of the FMD on the microbiome and metabolome in UC. *We hypothesize that the FMD plus biologic medications will improve dysbiosis by changing bacterial diversity and relative abundance of major bacterial groups compared to the patients' baseline and compared to patients receiving biologic medications alone. We further hypothesize that these changes will affect the production of bioactive metabolites.*

Exploratory Aim 3B. To identify patterns in the microbiome and metabolome that could predict clinical response to the FMD in combination with biologic medications. Our study is not powered to detect predictors of response to therapy but this exploratory aim will be a hypothesis generating experiment to determine if we identify microbiome/metabolome changes that could be used as predictors of response to FMD+ biologic medications A prior study examining microbiome predictors of response to vedolizumab found that there was a higher abundance of butyrate

producers at baseline in therapy-responsive CD patients[44]. *Therefore, we hypothesize that there will be baseline differences in the abundance and diversity of the microbiome composition and also in metabolites produced between those who respond to FMD intervention compared to those who do not respond.* Results from this preliminary analysis will be used to design future large validation cohorts.

Anticipated outcomes and future directions Aim 3a and 3b: We predict we will see an increase in alpha-diversity in patients receiving FMD plus biologic medications compared to biologic medications alone. We also expect an increase in diversity, and a shift to increase *Bacteriodetes* over *Firmicutes* as was seen in the initial FMD studies in healthy participants [12]. Based on prior studies, we expect to find a higher abundance of butyrate producers at baseline in therapy-responsive UC patients. In the future, we want to drill down to whether a particular microbiome predicts clinical response of a patient with UC to FMD. To that end, future studies will use stool from patients with clinical/biochemical benefit to FMD versus those that have not had a benefit to "humanize" germ-free mice. Humanizing germ-free mice with microbiota from patients has been used to better understand the functional aspects of the microbiota that sequencing analyses do not provide [45,46] Our investigative team has experience with these studies and we do not expect any technical issues.
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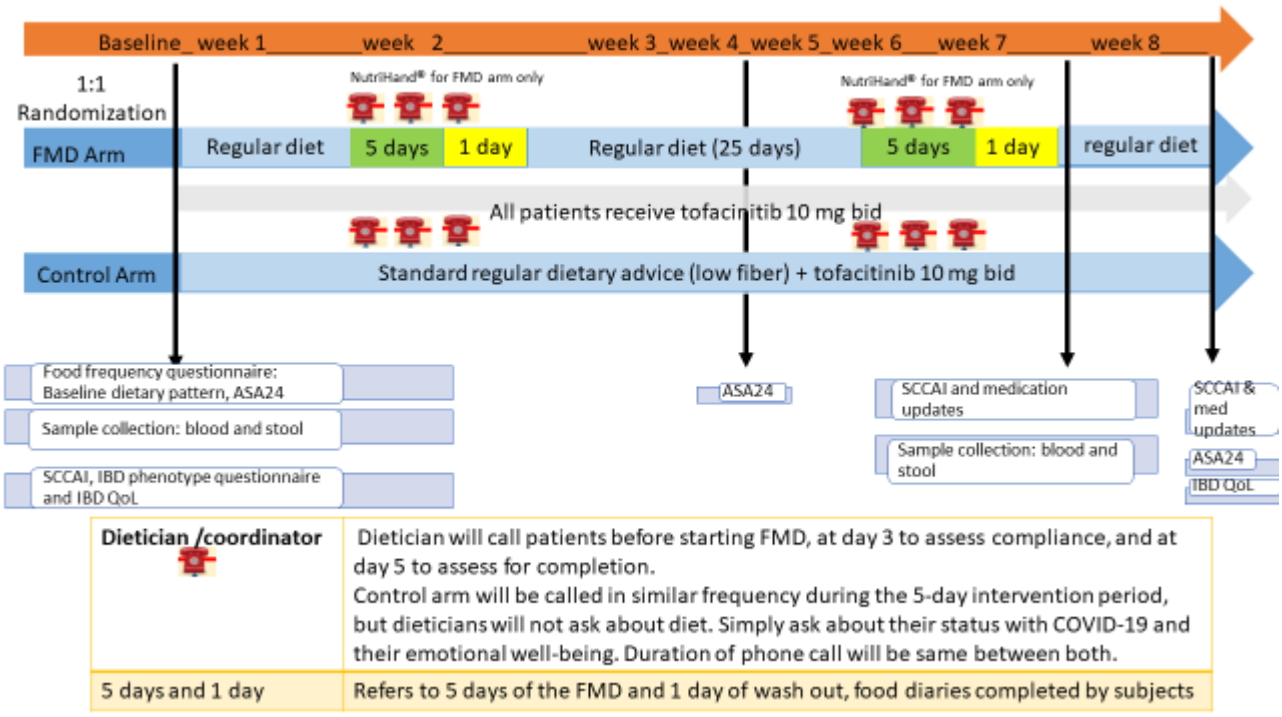
10) **Procedures Involved***

We will evaluate the effect on clinical response of adding two FMD intervals into standard biologic medication induction. Eligible participants (*see inclusion criteria*) will be randomly assigned to either Arm 1 (2 intervals of FMD + biologic medication at standard dose of induction) or Arm 2 of the study (standard low fiber dietary recommendations + biologic medication at standard dose of induction); Figure 1. Both arms will have initial baseline assessments including a detailed IBD questionnaire querying disease activity and severity, as well as a "short" IBD Quality of Life questionnaire. Baseline serum CRP and stool for measurement of fecal calprotectin, microbiome and metabolites to be repeated again in both arms 2 days after completion of the last FMD interval (week 7, Thursday). Additionally, we will measure levels of ketosis in participants of the FMD arm for indications of fasting. If not possible we will arrange with a local lab to collect samples and send them with an overnight currier. We will perform the second sample collection 2 days after finishing the last FMD based on prior methods evaluating microbiome changes from the FMD [29]. A last assessment of clinical response, IBD questionnaires, and medication updates will be performed at the end of the week 8 induction period. *Randomization:* We will perform randomization using a random allocation sequence that will be prepared online (www.stattrek.com), a statistical and analytical tool that uses block randomization with a 1:1 ratio of FMD to standard low fiber diet. Participants will be blinded to the diet allocation and informed that both diets could change the amount of protein, carbohydrates and fat intake from their regular diet and restrict caloric intake for max 5 days but that one diet is under investigation and the other is a standard diet provided to patients.

Figure 1. see below, next page



Dietician /coordinator	Dietician will call patients before starting FMD, at day 3 to assess compliance, and at day 5 to assess for completion. Control arm will be called in similar frequency during the 5-day intervention period, but dieticians will not ask about diet. Simply ask about their status with COVID-19 and their emotional well-being. Duration of phone call will be same between both.
5 days and 1 day	Refers to 5 days of the FMD and 1 day of wash out, food diaries completed by subjects

Instruments for diet ascertainment: We will use the three following validated diet questionnaires:
1. The National Health and Nutrition Examination Survey (NHANES) Food Frequency Questionnaire (FFQ). The FFQ will be self-administered but guidance will be provided if needed by our staff nutritionist. Nutritional calculations will be performed by uploading the questionnaire results through the NCI website with diet* Calc v1.5.0.30. The study dietician will assist with these calculations and interpretation. We will measure baseline diet on all enrolled patients using a validated food-frequency questionnaire (FFQ) to measure diet intake over the last year. Participants will be asked to complete FFQ at baseline.

2. Automated Self Administered-24 (ASA-24) is a validated nutritional questionnaire that queries food intake over the last 24 hour period.[31] The ASA24 will be self-administered but guidance will be provided if needed by our staff nutritionist. Participants will be asked to complete an ASA24 recall at baseline, between week 4 and 5, and a third time at week 8 (end of study).

3. NutriHand® is a web-based food diary online tool. Their services allow planning, tracking, analyzing and reporting on meals and medical information. Only participants in the FMD arm will be required to enter their daily food intake and only during the fasting mimicking diet weeks, 2 and 5. NutriHand® will be self-administered but guidance will be provided if needed by our staff nutritionist.

The Fasting Mimicking Diet: FMD and low fiber diet advice will be provided to all participants by the same research dietician with extensive training in IBD counseling before the start of the intervention and will last approximately 20 minutes. In order to standardize and ensure appropriate intake of the FMD, we will purchase commercially available kits of the FMD and provide them to patients one week before each planned interval (at study recruitment and again at week 5, just prior to

initiation of the second FMD at week 6) (Fig 1) [32]. The kits will be purchased through L-Nutra , their *Prolon*® 5-day meal plan include foods such as soups, snacks, bards, teas and supplements (omega-3 fatty acids) for a total of 66 items per box. The diet is plant-based, gluten free and dairy free without any artificial preservatives, chemicals or biologically active ingredients. Participants in the FMD arm will also be provided with a "Tips and Tricks" pamphlet for additional nutritional guidance. Patients will be instructed to maintain water intake constant in both arms, to 8 glasses a day. On Day 6, after the FMD, users will be instructed to avoid binge eating and resume their normal diet gradually. They should start with liquid foods, such as soups and fruit juices, followed by light meals, including rice, pasta and small portions of meat, fish and/or legumes. A normal diet can be resumed on day 7, or 24 hours after the FMD interval as per protocol from prior FMD studies [33]. To monitor adherence, participants will be instructed to record everyday all the food they eat in a food diary (NutriHand®) or take pictures of their food and email them to a secure email address. The email address will be created solely for the purpose of this study through the University of Miami Information Technology Department and will be registered under the Univeristy's secure server. The dietician will monitor the NutriHand® provider website and the email inbox daily to ensure foods are being recorded, if food is not recorded, the dietician takes immediate action and calls the patient.

11) **Data and Specimen Banking***

During the course of the clinical interventions in Aim 1, we will collect serum and stool (baseline and week 7) to microbiome/metabolome changes (Figure 1). Patients in the study will collect a morning stool sample and bring it to the clinic on ice, or an arranged FexEx shipping method. This stool will be aliquoted in an anaerobic chamber as to not disturb the anaerobic flora, snap frozen, and stored at -80^{O}C the same day until analysis of microbiome/metabolome changes. Storage will be done at the Abreu lab (see LOS). We have chosen to perform metagenomic shotgun sequencing of stool to identify specific microbes and also abundance of microbial genes including microbial enzymes. This will provide insight into the microbiota beyond the superficial description of bacterial community structure using 16S sequencing. Furthermore, we will pair metagenomic sequencing with metabolomic profiling to identify host-microbial interaction and downstream actionable targets.

Total genomic DNA will be isolated from one frozen aliquot using QIAamp DNA Stool Mini Kit (Qiagen) in Dr. Abreu's laboratory. Genomic DNA will then be sent to the University of Minnesota Genomics Core Facility for microbiome metagenomic sequencing. From the raw sequencing data, Dr. Ban from the BCC Core will utilize curated genome databases and a high-performance data-mining algorithm that rapidly disambiguates hundreds of millions of metagenomic sequence reads into the discrete microorganisms engendering the particular sequences. Overall classification precision is maintained through aggregation statistics. The last aliquot will be sent to the Proteomics & Metabolomics Facility at the Wistar Institute for metabolomics analyses. We routinely use these 2 facilities for all our microbiome sequencing and metabolomics analyses. Briefly, polar metabolites are separated under HILIC pH 9 condition and analyzed using a Thermo Q-Exactive HFX mass spectrometer [42.] Metabolites identification and quantification will be performed using Compound Discoverer 3.0 from a list of more than 200 verified compounds. Additionally, MS/MS data fragmentation and database will be searched against the mzCloud library (mzcloud.org) for potential identification and quantification of metabolites with a minimum score of 50,70,71. The raw data set will be transformed into principal components (PC), such as principal component analysis (PCA), orthogonal partial least square discriminant analysis (OPLS-DA), or partial least square- discriminant analysis (PLS-DA) [43]

12) **Data Management***

Power analysis: Given the lack of data on the influence of diet on induction of remission in UC, we used a diet study that measured the effect of diet on clinical remission in CD patients undergoing infliximab induction to conduct power analysis. Similar to the FMD, this diet was plant-based. The study reports that 96% of patients who followed the diet intervention in combination with infliximab had clinical remission compared to reported rates of clinical remission using IFX alone (64%) [33]. We calculate based on this study that approximately 76 patients in each arm (38 patients assigned to receive tofacitinib or other biologic medication plus FMD and 38 patients assigned to receive tofacitinib or other biologic medication only) would provide the trials with 80% power to detect a difference of 32 percentage points between the tofacitinib or other biologic medication + diet group and the tofacitinib or other biologic medication only group in the rates of the primary end points, assuming rates in the control arm of 64% for the primary end point. This would allow us to detect an alpha significance of 5%.

Aim 1 Analysis: All subjects will be included in the arm assigned regardless of treatment adherence (intention to treat). We will compare the percentage of patients who achieved clinical response among participants in Arm 1 to those in Arm 2 after 8 weeks, taking into account clinically significant covariates using multivariable logistic regression. We will also compare SCCAI as a continuous end point using multivariable linear regression. Secondary outcomes including improvement in fecal calprotectin and CRP will be evaluated as continuous outcomes, using multivariable linear regression, taking into account relevant covariates. Clinical categorical will be analyzed using chi-square or Fisher's Exact Test. Continuous variables will be analyzed using student's t-tests, ANOVA or Mann-Whitney U tests depending on their distribution. Statistical analyses will be performed using R studio.

Aim 2 Analysis: Prior studies derive a Western diet pattern using subset of 40 food groups. [9,36,37] This is done by factor analysis done to aggregate food groups based on degree of correlation between the food item and data set. We will conduct a confirmatory factor analysis (CFA) on the WD indicators and will compute factor scores for each patient using previously calculated factor loadings.36,37 A dietary pattern score is generated for each participant by summing weighted intakes of food groups, adjusted for energy consumption using the residual method.9,36,37 After construction of Western diet pattern scores for each patient, we will examine the association between scores and the clinical response, measured as a categorical and continuous variable (using SCCAI score, described in Aim 1). We will examine the effect of diet pattern (continuous) on clinical response (categorical) using multivariable logistic regression on each diet score, taking into account covariates mentioned in Aim 1.

Aim 3a/3b: Plan of analysis of microbiome and metabolite data: Our metabolomic data will be complemented with our microbiome and metadata, such as clinical parameters (change in SCCAI, fecal calprotectin, CRP) and diet to extrapolate a mechanistic model that will explain the microbial/metabolite community structure and function. Dr. Ban from our BCCC core, has collaborated with us on these types of data analyses from another similar study (publication pending). This integrative method for exploring such data is necessary to advance the understanding of the microbial metabolites and the microbial community. We will use this mechanistic model to examine which bacteria produce the metabolites but also which specific inflammatory markers (CRP/Fecal calprotectin) and clinical data link to this production. Using this computational analytical approach, we further hypothesize that we will see trends in clinical parameters which will allow us to correlate it to environmental cues in the human gut, such as microbiome and metabolites. To assess the

differences between the clinical parameters and clinical response, we will use permutational multivariate analysis of variance. Therefore, different parameters will be addressed in each experimental setup, and full permutation of the data will be done with Monte–Carlo tests (accounting for type III error), where the fixed effects sum to zero with 9,999 permutations. For the first experiment, the factors will be the SCCAI score and the presence/absence of clinical parameters. For the second test, the factors are each microbiome, metabolites and clinical parameters. For all the experiments, statistical comparisons will be performed using one-way ANOVA (Tukey's test) to compare pairwise differences between treatments.

13) **Provisions to Monitor the Data to Ensure the Safety of Subjects***

All data will be safeguarded in accordance with the Health Insurance Portability and Accountability Act (HIPAA) and the principles and practices of strict confidentiality. The interview and survey responses from formative evaluation will not be linked to identifying information. Data will be stored in a locked file cabinet and files will be stored on a password protected computer in a password protected database in the office of the PI. Participants will be provided a unique identifier to be used in data analysis. Last, no individuals will be identified in any presentations or publications resulting from this research. In such a way we are protecting participants from risks related to privacy and confidentiality.

14) **Withdrawal of Subjects***

Participation in this research study is voluntary. Participants can choose not to participate in this study either at the beginning or at any time during the study. There is no penalty nor any loss of benefits for participants. Participants will be compensated for completed study visits. Withdrawing will not have an adverse impact on participant's present or future health care. For their safety, participants will be asked to undergo a final evaluation visit. Participants can contact dietitian or study coordinator if they wish to withdraw from study.

Participants may be withdrawn from study without their consent if they are not able to follow dietary recommendations or if the participant does not tolerate biologic medication therapy. These cases will be carefully reviewed by the participant's physician.

All the information about the participant and the samples (blood, urine, saliva, or other samples) we obtain will be kept. This data will remain as part of the study database. Participants will also be asked if we can continue to review their medical record and collect data about their medical care in the future. If they agree to allow us to keep collecting data after withdrawing from the study, this new data will be handled the same as the other research data.

15) **Risks to Subjects***

Certain risks are associated with the participation in the study:
Participants may not like the particular dietary guidance given to them and they may not find dietary recommendations to their liking. Participants may also experience more hunger, mainly if their usual, baseline diet is higher in calories than the study diet.

The process of interviewing may cause distress and discomfort because will be talking about personal activities and thoughts. It is possible that some of the questions that are asked are similar or the same

as others asked by other health professionals and the participant may wonder why it is necessary to answer them again. It may be tiresome to you to be asked these questions again.
The investigator will also make a judgment about the ability to understand this consent form and the ability to participate in the interview.

In addition, patient may experience discomfort or fatigue in having the study doctors ask questions on the phone, in person, and with the questionnaires. The risks of answering questions are minimal, but the participant can stop at any point if the participant feel too tired to continue with the assessments.

The risks of blood drawing include: fainting, temporary pain, and/or bruising at the site of the needle stick. Rarely, infection or the formation of a small clot or swelling of the vein and surrounding area may occur. Although the samples used for the current study are coded, there is still a minimal risk of confidentiality breach. However, all the clinical and phenotypic data that can be linked to the samples is saved in UM password-protected databases and only authorized personnel can access them.

16) Potential Benefits to Subjects*

Subjects may not benefit directly from participating in this study, although it is possible that they experience improvement in their medical symptoms if the diet intervention is effective. However, this benefit may decrease or end once participation in the study ends and if they choose to go back to eating their regular diet.

17) Vulnerable Populations*

N/A, This research does not involve vulnerable populations.

18) Multi-Site Research*

This is not a multi-site study.

19) Community-Based Participatory Research*

This is not a community-based participatory research study

20) Sharing of Results with Subjects*

Most tests done on samples in this research study are only for research and have no clear meaning for a participant's health care. However, if the research with identifiable information or samples gives results that do have meaning for a participant's health, the researchers will contact the participant.

21) Setting

Recruitment for this study will take place at the following location:

- The Crohn's & Colitis Center/UMHC University of Miami Hospital & Clinics

Located on the +first floor +at 1475 N.W. 12th Avenue, Miami, Florida, 33136. Sylvester and UMHC are in connecting buildings on the northeast corner of the N.W. 12th Avenue and N.W. 14th Street intersection.

Phone: 305-243-UMGI (8644) option 3, option 4
Fax: 305-689-1852

22) Resources Available

Principal Investigator - Oriana M. Damas, M.D.,

Dr. Damas is Assistant Professor of Medicine and the Director of Translational Studies at the University of Miami. Dr. Damas has experience in translational studies and diet in inflammatory bowel disease (IBD). Her research focuses on the influence of diet and genetics on predictors of disease relapse in ulcerative colitis patients, ultimately with the hope of identifying patient-profile panels that will identify/predict what they will respond to. She will be in charge of the recruitment of patients and will be responsible for the overall project design, supervision, data analyses and generation of project reports and publications. Her mentor, Maria T. Abreu, will continue to be available to her for ideas, problem-solving, logistics and for grant writing/publications.

Clinical Research Coordinator

A CRC with preferably 2 years of experience as a Clinical Research Coordinator. Preferably trained in ascertainment protocols, procedures, and data management. Will help recruit patients for ongoing diet study. Will work closely with Dr. Damas in approaches to tracking adherence and maintaining high rates of follow-up. Will be responsible for regulatory and compliance application and maintenance. Will also assist with RedCap data entry during the length of the study.

Dietician

A dietitian with preferably 2 years of clinical and research experience. The dietician will preferably have extensive experience in diet education, Medical Nutrition Therapy and evaluation of nutritional outcome. In addition, experience on healthy lifestyle intervention programs and nutrition counseling software is preferred. The dietician is in charge of the nutritional assessment and description of the diets before and during diet intervention of the recruited patients. Dietician's bulk effort in years 1-3 will be dedicated to ensuring patients are adhering to dietary interventions (total of 10 days per patient) and that patients maintain a low residue diet in the control arm. The dietician will also oversee daily food diary monitoring during FMD intervals and corrective action necessary to maintain adherence to the diet.

Bioinformatician (10% effort, year 3)

A bioinformatician with preferably a PhD in computational science and statistics and experience. The bioinformatician will focus on the statistical associations between all the data collected during this proposal.

23) Prior Approvals

N/A

24) Local Number of Subjects

76 subjects will be enrolled in this study in order to complete the research procedures. The total number eligible subjects to be screened is unknown.

25) Confidentiality

Check all that apply:

☒ Data obtained or created for this research will be stored on an encrypted electronic device or system owned by the University of Miami or on a cloud storage system that has been approved by the University of Miami for storage or research data.

☐ The Investigator (or research staff) will record (e.g. write down, abstract) data collected in a manner that **does not include** any indirect or direct identifiers and the recorded data **will not be linked to the individual's' identity.**

☒ The investigator (or research staff) will record (e.g. write down, abstract) the data collected in a manner that does not include any direct identifiers of the subject. The investigator **will assign a code to each subject and link the code to the subject's identity**. The research team will maintain the link to the subject's identity on a document separate from the research data. Both documents will be stored in separate files on a University of Miami encrypted device or on a University of Miami approved cloud storage system. The research team will destroy the identifiers at the earliest opportunity.

☒ The research team will maintain the research data for at least three years.

☐ *Bio*-Specimens obtained for this research will be stored without any direct or indirect identifiers.

☒ *Bio*-Specimens obtained for this research will be stored in a de-identified coded manner.

☒ When required to transport data or bio-specimens for this research, the research team will transport the data and bio-specimens in a de-identified (or anonymous) manner with a link to the individual subject's identity maintain separately from the data and/or bio-specimen.

26) Provisions to Protect the Privacy Interests of Subjects

The research team will inform participants that optimum measures will be taken to limit the use or disclosure of their personal information, including information from this research study and from their medical records, to people who have a need to review this information.

Subjects will also be informed of the following privacy protection provisions that will be made:

- We will remove identifiable information from data we collect
- After we remove all of the identifiers, we will place a code on the information. The code will be linked to participant identity, but the link will be kept in a location that is separate from study data.
- We will maintain individual study data on encrypted computers and access to the information will be limited to only members of the research team who need the access to properly conduct the study.
- The information we send to the sponsor will not include information that directly identifies participants. Instead, a code will be applied to the data and link between the code and individual identity will be kept at the research site.
- Any information we publish will not identify participants directly. Identifiable information may be removed from the data or samples we collect, only then will biosamples be used for this study and any future study
- The Research Team will make every effort to follow-up with participants and be available to answer questions/concerns

27) Compensation for Research-Related Injury

Although risks are unlikely, if injury should occur, treatment will in most cases be available. A partcipant's insurance company may or may not pay for these costs. If a participant does not have insurance, or if their insurance company refuses to pay, the sponsor will pay for the cost of treating the injury. The University of Miami and the sponsor are not planning to pay for pain, lost wages, and other costs that may incur if a participant is injured due to the research. However, participants do not give up any legal rights to obtain payment for an injury by after they sign the consent form. This policy does not prevent them from trying to obtain compensation through the legal system.

28) Economic Burden to Subjects

N/A

29) Consent Process

Informed consent will be done in all patients. The documentation will be attached to the protocol.

Non-English Speaking Subjects

Other than English, consent forms and study-related questionnaires will be made available in Spanish to participants whose primary language is Spanish, so prospective subjects understand the study.

30) Process to Document Consent in Writing

This study will be following "SOP: Written Documentation of Consent (HRP-091).

The consent process will be initiated with study participants prior to starting any research procedures. Participants will be given ample time to consider their agreement. A member of the study team will be available to answer any questions throughout the length of the study

time. No one in a perceived coercive position in relation to the participant will engage in the consenting process. Consent will be obtained voluntarily prior to initiating any study procedures. Written consent will be obtained from every patient prior to initiation of the study. Consents will be provided in both English and Spanish. No minors will be enrolled in this study.

During the clinic visit, the consent form will be reviewed with the prospective study subject, and the investigator will be available to answer questions regarding procedures, risks, and alternatives. If subject is willing to participate, a clinic visit will be scheduled for screening process where CRC will obtain written informed consent from each subject. Consent will be obtained before any protocol-specific procedures are performed. Documentation of the subject's informed consent for and participation in this study will be noted in the subject's medical record. If the subject is enrolled in a sub study of this protocol, a sub study-specific consent must also be used. The subject representative must be provided with a copy of the consent form for the main study and a copy of any separate consent form for the sub study (if applicable).

31) Authorization for Use and Disclosure of Protected Health Information (HIPAA)

If the research team will access patient medical records or other identifiable health information for this research, you must obtain a waiver of the requirement for written authorization from the patients to access their medical records.

Type of Request:

☒ Waiver of Authorization for access to medical record for subject identification/recruitment.

☐ Waiver of Authorization for access to medical record to obtain data for the research.

Confirm that you will destroy or de-identify the information you collect at the earliest opportunity.

☒ ***I confirm***

Confirm that the information you collect will not be reused or disclosed to any other person or entity, except as required by law, for authorized oversight of the research study or for other research for which the use or disclosure of PHI is permissible. ☒ ***I confirm***

32) Drugs or Devices

Pfizer will ship tofacitinib product in labeled bottles with an identifying code. Pfizer will bottle with a specific tablet count based on the protocol/dosing (i.e., 60 tablets in each bottle for 1 month supply) and the site would be responsible for labelling the bottles and dispensing. The tofacitinib pills will not

have any identifying markings. Since the study is single blind, both arms are receiving tofacitinib 10 mg.

Remote Consent & Authorization

Based on FDA Guidance on the Conduct of Clinical Trials of Medical Products during the COVID-19 Public Health Emergency, the following will be implemented during times of quarantine when in-person, face-to-face contact is not possible.

Eligible patients will be consented via Zoom for Healthcare or through an arranged three-way call with the potential participant, an impartial witness, and if desired and feasible, additional people requested by the participant, (e.g. next of kin).

Once the study coordinator arranges the time and communication method for the consent process for a new or existing participant, or for re-consent, the study coordinator will:

1. Send a copy of the consent document via secure email or U.S. Mail.
2. Arrange for a witness to attend and witness the consent discussion.
3. Let the participant know that a witness will join the consent meeting.
4. Set up a Zoom meeting or 3-way call and send an invitation to the attendees.

During the remote meeting the study coordinator or the person obtaining consent will:

1. Identify everyone on the call.
2. Review the informed consent with the participant, answer the participant's questions and ask questions of the participant to confirm comprehension.
3. Ask the participant if s/he consents to participate/continue participation.
4. If the participant agrees, ask him/her to sign and date the consent document. If using 3-way call, ask the participant to confirm s/he signed & dated the document.
5. Ask the participant to scan or take a picture of each page of the documents and email the signed/dated documents to the study team.
6. If the participant is unable to take a picture, document the circumstances.
7. The person conducting the consent process should sign and date a copy of the consent document.
8. The witness should sign & date on the witness line of a copy of the consent document.

Documenting the Remote Consent Process

The study coordinator or the person conducting the consent process should document the purpose for the remote consent (COVID-19), and each step of the process. A printed copy of the informed consent document signed by the participant, investigator and witness will be placed in the participant's research record.

Additionally, the study coordinator or the person obtaining consent will document how s/he confirmed that the patient consented and signed the consent form. The note should include a statement indicating why the informed consent document signed by the participant was not retained, (e.g., due to contamination of the document by infectious material.) If the participant cannot send a picture of the signed document, the person obtaining consent should document why a copy of the signed document is not available. The note will explain why the research team does not have the signed and dated document.

References

1. Ordas, Eckmann L, Tlamini M,Baumgart DC, Sandborn WJ. Ulcerative Colitis. The Lancet (2012). Nov 3;380(9853):1606-19. doi: 10.1016/S0140-6736(12)60150-0
2. Sandborn WJ, Su C, Sands BE, et.al. Tofacitinib as Induction and Maintenance Therapy for Ulcerative Colitis. The New England Journal of Medicine 2017 May 4;376(18):1723-1736. doi: 10.1056/NEJMoa1606910.
3. Limketkai BN, Iheozor-Ejiofor Z, Gjuladin-Hellon T, et al. Dietary interventions for induction and maintenance of remission in inflammatory bowel disease. Cochrane Library. 2019 Feb 8;2:CD012839. doi: 10.1002/14651858.CD012839.pub2.
4. Candy S, Borok G, Wright JP, Boniface V, Goodman R. The value of an elimination diet in the management of patients with ulcerative colitis. S Afr Med J. 1995 Nov;85(11):1176-9.
5. Chiba M, Tsuji T, Nakane K, et. al. Induction with Infliximab and a Plant-Based Diet as First-Line (IPF) Therapy for Crohn Disease: A Single-Group Trial. The Permanente Journal.2017;21:17-009. doi: 10.7812/TPP/17-009.
6. D'Haens G, Baert F, van Assche G , et al. Early combined immunosuppression or conventional management in patients with newly diagnosed Crohn's disease: an open randomised trial. The Lancet 2008 Feb 23;371(9613):660-667. doi: 10.1016/S0140-6736(08)60304-9.
7. Loftus EV Jr. Update on the Incidence and Prevalence of Inflammatory Bowel Disease in the United States. *Gastroenterol Hepatol (N Y)*. 2016;12(11):704–707.
8. Ananthakrishnan, A. N., Khalili, H., Song, M., Higuchi, L. M., Richter, J. M., Nimptsch, K., & Chan, A. T. (2015). High school diet and risk of Crohn's disease and ulcerative colitis. Inflammatory bowel diseases, 21(10), 2311-2319.
9. Ananthakrishnan AN, Khalili H, Konijeti GG, et al. Long-term intake of dietary fat and risk of ulcerative colitis and crohn's disease. Gut. 2014;63(5):776-784.
10. Racine A, Carbonnel F, Chan SS, Hart AR, Bueno-de-Mesquita HB, Oldenburg B, van Schaik FD, Tjønneland A, Olsen A, Dahm CC, Key T. Dietary patterns and risk of inflammatory bowel disease in Europe: results from the EPIC study. Inflammatory bowel diseases. 2015 Dec 30;22(2):345-54.
11. Afzali, Anita, and Raymond K. Cross. Racial and ethnic minorities with inflammatory bowel disease in the United States: a systematic review of disease characteristics and differences. Inflammatory Bowel Diseases 22.8 (2016): 2023-2040
12. Rangan P, Choi I, Wei M, et al. Fasting Mimicking Diet Modulates Microbiota and Promotes Intestinal Regeneration to Reduce Inflammatory Bowel Disease Pathology. Cell Rep. 2019 Mar 5;26(10):2704-2719.e6. doi: 10.1016/j.celrep.2019.02.019.
13. Cabo de Rafael, Mark P.Mattson. Effects of Intermittent Fasting on Health, Aging, and Disease. The New England Journal of Medicine.2020 Jan 16;382(3):298. doi:10.1056/NEJMx190038.
14. Betteridge JD, Armbruster SP, Maydonovitch C, Veerappan GR. Inflammatory bowel disease prevalence by age, gender, race, and geographic location in the US military health care population. Inflammatory bowel diseases. 2013 Jun 1;19(7):1421-7.
15. Wu, G. D. et al. Linking long-term dietary patterns with gut microbial enterotypes. Science (New York, N.Y.) 334, 105-108, doi:10.1126/science.1208344 (2011).

16. Ni, J., Wu, G. D., Albenberg, L. & Tomov, V. T. Gut microbiota and IBD: causation or correlation? Nature reviews. Gastroenterology & hepatology 14, 573-584, doi:10.1038/nrgastro.2017.88 (2017)
17. Devkota, S. et al. Dietary-fat-induced taurocholic acid promotes pathobiont expansion and colitis in Il10- /- mice. Nature 487, 104-108, doi:10.1038/nature11225 (2012)
18. David, L. A. et al. Diet rapidly and reproducibly alters the human gut microbiome. Nature 505, 559-563, doi:10.1038/nature12820 (2014)
19. Huda-Faujan, N. et al. The impact of the level of the intestinal short chain Fatty acids in inflammatory bowel disease patients versus healthy subjects. The open biochemistry journal 4, 53-58, doi:10.2174/1874091x01004010053 (2010)
20. Damman, C. J., Miller, S. I., Surawicz, C. M. & Zisman, T. L. The microbiome and inflammatory bowel disease: is there a therapeutic role for fecal microbiota transplantation? The American journal of gastroenterology 107, 1452-1459, doi:10.1038/ajg.2012.93 (2012).
21. Vaughn, B. P. et al. Increased Intestinal Microbial Diversity Following Fecal Microbiota Transplant for Active Crohn's Disease. Inflammatory bowel diseases 22, 2182-2190, doi:10.1097/mib. (2016).
22. Sun, M., Wu, W., Liu, Z. & Cong, Y. Microbiota metabolite short chain fatty acids, GPCR, and inflammatory bowel diseases. Journal of gastroenterology 52, 1-8, doi:10.1007/s00535-016-1242-9 (2017).
23. D'Souza, W. N. et al. Differing roles for short chain fatty acids and GPR43 agonism in the regulation of intestinal barrier function and immune responses. PloS one 12, e0180190, doi:10.1371/journal.pone.0180190 (2017)
24. Wu, G. D. The Gut Microbiome, Its Metabolome, and Their Relationship to Health and Disease. Nestle Nutrition Institute workshop series 84, 103-110, doi:10.1159/000436993 (2016)
25. Bloom, S. M. et al. Commensal Bacteroides species induce colitis in host-genotype-specific fashion in a mouse model of inflammatory bowel disease. Cell host & microbe 9, 390-403, doi:10.1016/j.chom.2011.04.009 (2011).
26. De Filippo, C. et al. Impact of diet in shaping gut microbiota revealed by a comparative study in children from Europe and rural Africa. Proceedings of the National Academy of Sciences of the United States of America 107, 14691-14696, doi:10.1073/pnas.1005963107 (2010).
27. Ni, J. et al. A role for bacterial urease in gut dysbiosis and Crohn's disease. Science translational medicine 9, doi:10.1126/scitranslmed.aah6888 (2017)
28. Blasbalg TL, Hibbeln JR, Ramsden CE, Majchrzak SF, Rawlings RR. Changes in consumption of omega-3 and omega-6 fatty acids in the United States during the 20th century. The American journal of clinical nutrition. 2011 May 1;93(5):950-62.
29. Davis N., Schechter CB, Ortega F., et al. Dietary patterns in blacks and Hispanics with diagnosed diabetes in New York City's South Bronx. Am J Clin Nutr. 2013; 97:878-85.
30. Sandborn, W. J., Su, C., Sands, B. E., D'Haens, G. R., Vermeire, S., Schreiber, S., & Friedman, G. (2017). Tofacitinib as induction and maintenance therapy for ulcerative colitis. New England Journal of Medicine, 376(18), 1723-1736.
31. National Health and Nutrition Surveys NHANES Nutrient, National Cancer Institute, epi.grants.cancer.gov/diet/usualintakes/ffq , Division of Cancer Control and Population Sciences.

32. The ProLon Fasting Mimicking Diet is the only dietary program patented for promoting longevity and healthspan. Prolon https://prolonpro.com/. Published February 2017.
33. Wei M, Brandhorst S, Shelehchi M, et.al. Fasting-mimicking diet and markers/risk factors for aging, diabetes, cancer, and cardiovascular disease. Science Translational Medicine. 2017 Feb 15;9(377). pii: eaai8700. doi: 10.1126/scitranslmed.aai8700.
34. Durchschein F, Petritsch W, Hammer HF. Diet therapy for inflammatory bowel diseases: The established and the new. World Journal of Gastroenterology. 2016 Feb 21;22(7):2179-94. doi: 10.3748/wjg.v22.i7.2179.
35. Owczarek D, Rodacki T, Domagała-Rodacka R, Cibor D, Mach T. Diet and nutritional factors in inflammatory bowel diseases. World Journal of Gastroenterology 2016 Jan 21;22(3):895-905. doi: 10.3748/wjg.v22.i3.895.
36. Chilton FH, Murphy RC, Wilson BA, et al. Diet-gene interactions and PUFA metabolism: A potential contributor to health disparities and human diseases. Nutrients. 2014;6(5):1993-2022.
37. Chilton FH, Dutta R, Reynolds LM, Sergeant S, Mathias RA, Seeds MC. Precision Nutrition and Omega-3 Polyunsaturated Fatty Acids: A Case for Personalized Supplementation Approaches for the Prevention and Management of Human Diseases. Nutrients. 2017 Oct 25;9(11):1165.
38. Wei M, Brandhorst S, Shelehchi M, et al. Fasting-mimicking diet and markers/risk factors for aging, diabetes, cancer, and cardiovascular disease. Science Translational Medicine. 2017 Feb 15;9(377). pii: eaai8700. doi: 10.1126/scitranslmed.aai8700.

37. Sandborn, W. J., Su, C., Sands, B. E., D'Haens, G. R., Vermeire, S., Schreiber, S.,

.& Friedman, G. (2017). Tofacitinib as induction and maintenance therapy for ulcerative colitis. New England ournal of Medicine, 376(18), 1723-1736

39. David, L. A. et al. Diet rapidly and reproducibly alters the human gut microbiome. Nature 505, 559-563, doi:10.1038/nature12820 (2014).
40.Dolan, K. T. & Chang, E. B. Diet, gut microbes, and the pathogenesis of inflammatory bowel diseases. Molecular nutrition & food research 61, doi:10.1002/mnfr.201600129 (2017).
41.Walker, A. W. et al. Dominant and diet-responsive groups of bacteria within the human colonic microbiota. The ISME journal 5, 220-230, doi:10.1038/ismej.2010.118 (2011).
42. Lucas, C. J., Galettis, P. & Schneider, J. The pharmacokinetics and the pharmacodynamics of cannabinoids. *Br J Clin Pharmacol* 84, 2477-2482, doi:10.1111/bcp.13710 (2018).
43. Atsmon, J. *et al.* PTL401, a New Formulation Based on Pro-Nano Dispersion Technology, Improves Oral Cannabinoids Bioavailability in Healthy Volunteers. *J Pharm Sci* 107, 1423-1429, doi:10.1016/j.xphs.2017.12.020 (2018).
44. Ananthakrishnan AN, Luo C, Yajnik V, et. al.Gut Microbiome Function Predicts Response to Anti-integrin Biologic Therapy in Inflammatory Bowel Diseases. Cell Press 2017 May 10;21(5):603-610.e3. doi: 10.1016/j.chom.2017.04.010.
45. Torres, J. *et al.* Infants born to mothers with IBD present with altered gut microbiome that transfers abnormalities of the adaptive immune system to germ-free mice. *Gut* 69, 42-51, doi:10.1136/gutjnl-2018-317855 (2020).
46. Britton, G. J. *et al.* Microbiotas from Humans with Inflammatory Bowel Disease Alter the Balance of Gut Th17 and RORgammat(+) Regulatory T Cells and Exacerbate Colitis in Mice. *Immunity* 50, 212-224.e214, doi:10.1016/j.immuni.2018.12.015 (2019)