| | |
|---|---|
| Title: | **Biological and Immunomodulator Monotherapy Withdrawal in Pediatric Crohn Disease Patients with Deep Clinical Remission Using the Crohn Disease Exclusion Diet** |
| Short Title | CEASE |
| Sponsor: | CHOP IBD Center |
| eIRB Number: | IRB 14-011628 |
| Protocol Date: | March 30, 2015 |

Amendment 1 Date: May 27, 2015

Amendment 2 Date: June 15, 2015

Amendment 3 Date: May 17, 2016

Amendment 4 Date: November 30, 2016

Amendment 5 Date: February 3, 2017

Amendment 6 Date: April 11, 2018

Amendment 7 Date: May 10, 2019

**PI: Ronen Stein, MD**
Division of Pediatric Gastroenterology
The Children's Hospital of Philadelphia
3401 Civic Center Blvd, Philadelphia, PA 19104
phone: 215-590-7801
SteinR2@email.chop.edu

## TABLE OF CONTENTS

## ABBREVIATIONS AND DEFINITIONS OF TERMS

| | |
|---|---|
| CD | Crohn disease |
| PCDAI | Pediatric Crohn Disease Activity Index |
| CDED | Crohn's Disease Exclusion Diet |
| PEN | Partial enteral nutrition |
| TNF- α | Tumor necrosis factor alpha |
| CRP | C-reactive protein |
| ESR | Erythrocyte sedimentation rate |
| MARS | Medication Adherence Rating Scale |

## ABSTRACT

Context:

Crohn disease (CD) is an idiopathic, chronic, relapsing and remitting inflammatory condition of the gastrointestinal tract with a high risk for complications and need for surgical interventions. The incidence rate in the pediatric population is rapidly increasing. Immunomodulators and biologic therapies are effective at inducing and maintaining remission in pediatric CD, yet there is no proven strategy for reducing exposure to medications once sustained remission has been achieved. Diet has been proven to impact disease activity in CD and may allow for sustained drug-free remission.

Objectives: The primary objective of this study is to determine differences in time-to-relapse and the proportion of subjects with sustained relapse-free remission between subjects on the Crohn's Disease Exclusion Diet (CDED) and those on an unrestricted diet. The secondary objective is to determine the proportion of all subjects with sustained relapse-free remission 52 weeks after withdrawal of biological or immunomodulator monotherapy by the primary gastroenterologist as part of clinical care.

The third objective is to assess the compliance of subjects to the CDED.

Study Design: This will be an open-label, non-randomized pilot study involving 25 pediatric Crohn disease patients in deep clinical remission, who will be withdrawing from biologic or immunomodulator monotherapy based on the clinical judgment of their primary gastroenterologist. Subjects will choose whether or not to adhere to the diet. Subjects who do not adhere to the CDED will serve as controls.

Setting/Participants: Recruit outpatients with CD who will be withdrawing from biologic or immunomodulator monotherapy based on the clinical judgment of their primary gastroenterologist. Any patient in deep clinical remission who meets inclusion criteria and consents is eligible for this study.

Study Interventions and Measures: The study intervention is the CDED. Measures will be obtained from stool testing, diet logs/adherence questions, and medical record review.

Main study outcome measures: 1) Differences in time-to-relapse and proportion of subjects with sustained relapse-free response among those who do and do not participate in the dietary intervention as measured by the Pediatric Crohn's Disease Activity Index (PCDAI), C-Reactive Protein (CRP), microbiome, and stool calprotectin. 2) Compliance with the dietary intervention as measured by the modified MARS questionnaire.

## SCHEDULE OF STUDY PROCEDURES

| Parameter | Week 0 | Week 1 (Phone Call)[0] | Week 3 (Email) | Week 6 | Week 7 (Phone Call)[0] | Week 9 (Email) | Week 12 | Week 13 (Phone Call)[0] | Week 24 | Week 36 | Week 52 | Clinical Flare[00] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Inclusion/ Exclusion** | X | | | | | | | | | | | |
| **Stool Calprotectin** | X[+] | | | X | | | X | | X | X | X | X |
| **CRP, ESR*** | X | | | X | | | X | | X | X | X | X |
| **Weight*** | X | | | X | | | X | | X | X | X | |
| **Modified MARS questionnaire** | | X | | X | X | | X | X | X | X | X | X |
| **3 days food diary** | | | X | | | X | | | X | | | |
| **24-Hour Recall** | X | X | | X | | | X | | | X | X | X |
| **Tolerability** | | X | | | X | | | X | | | | |
| **CDED Phase 1 diet** | X | X | X | | | | | | | | | |
| **CDED Phase 2 diet** | | | | X | X | X | | | | | | |
| **CDED Phase 3 diet** | | | | | | | X | X | | | | |
| **CDED Phase 4 diet** | | | | | | | | | X | X | X | |
| **PCDAI*** | X | | | X | | | X | | X | X | X | X |
| **Stool for microbiome** | X | | | X | | | | | | | X | X |
| **Swabs (Rectal and Oral) for microbiome** | X | | | X | | | | | | | X | X |

*From Chart Review [+]From Chart Review (obtained within the previous 3 months)

[0]Only Subjects on study diet

[00] This study visit may occur during the first 52 weeks of the study if the subject is in the office for a clinical flare.

# 1 BACKGROUND INFORMATION AND RATIONALE

## 1.1 Introduction

Crohn disease (CD) is an idiopathic, chronic, relapsing and remitting inflammatory condition of the gastrointestinal tract with a high risk for complications and need for surgical interventions. The incidence rate of CD is estimated to be 1 in 10,000 children per year and is rising [1]. In children, CD tends to have a more aggressive course with more associated complications. The majority of children require an effective maintenance medication therapy to prevent relapse and complications.

To date, there is no proven strategy for reducing exposure to medication once sustained remission has been achieved. It is possible that medications cannot be easily stopped because environmental triggers that perpetuate the disease, such as diet, still remain. Akin to the connection between smoking and lung disease, if the environmental exposure continues the disease may continue to progress. In this proposed study, a dietary intervention, the Crohn's Disease Exclusion Diet (CDED) will be investigated.

## 1.2 Crohn's Disease Exclusion Diet

The CDED is a diet that excludes food felt to be pro-inflammatory, such as those that contain dairy, wheat products, sauces, soft drinks, fruit juice, processed meats dairy, and emulsifiers. It has previously been shown to be effective in inducing remission in patients with CD [2].

The following table details general categories of allowed and restricted foods in the diet:

| Allowed Foods | Restricted Foods |
|---|---|
| Fresh Fish and Chicken Breast (unlimited) | Processed or smoked meats |
| White Rice, Rice Noodles | Breads, Pastas, Cereals and Baked Goods |
| Fresh fruits | Dried Fruits |
| Fresh Vegetables | Dairy Products |
| Fresh Beef (limited amount) | Canned Food |
| Honey, Fresh Herbs, Onion Garlic, Olive and canola oil (for cooking). Limited table sugar | Packaged Snacks and candy |
| Water, Seltzer Water, Herbal Teas, Freshly-squeezed orange juice (limited) | Soft drinks, fruit juices, sweetened beverages, alcoholic beverages, coffee |

The diet is most restrictive during the first 6 weeks (CDED Phase I), then becomes less restrictive during the subsequent 6 weeks (CDED Phase II). There is no access to free diet during the first 12 weeks, but thereafter, as long as patients are otherwise compliant 1-2 free diet meal per week are allowed (CDED Phases III-IV). This free diet meal does allow a regular portion of dairy, wheat products, sauces, soft drinks, fruit juice, cakes, and ice cream, but does not allow processed meats such as hot dogs or luncheon meats and does not allow binging on non-recommended foods.

Because the diet does restrict some common foods, patients may need to take a caloric supplement of their choosing if they cannot meet nutritional and caloric needs with the diet

alone. The intention of any supplement will be to sustain caloric and nutritional balance, not to diagnose, mitigate or treat the underlying disease. The choice of supplementary formula will be based on patient preference, and is not part of the research study objectives. The diet does restrict many forms of calcium, so patients will need to take calcium supplementation to meet basic dietary requirements while on the CDED.

Sigall-Boneh et al. showed that the CDED achieved remission in 70% of 47 patients. Patients in remission had a significant improvement in markers of disease activity, including erythrocyte sedimentation rate (ESR), CRP, serum albumin, and the Pediatric Crohn Disease Activity Index (PCDAI). Among 18 patients practicing prolonged dietary restriction (range 6-24 months) with follow up colonoscopies, 14 patients had complete mucosal healing.

### 1.3 Relevant Literature and Data

The goals of therapy in pediatric CD are to induce and maintain clinical remission with the least toxicity, thereby improving quality of life and preventing known complications of CD, such as growth failure, bowel stricturing, or fistula formation. There are several broad categories of maintenance therapies in CD. Anti-inflammatory medications include 5-ASA drugs, antibiotics and non-conventional glucocorticoids. The immunomodulators include 6-mercaptopurine, azathioprine, and methotrexate. The biologics form another class of medications that more directly target pro-inflammatory cytokines. Infliximab and adalimumab are two commonly used biologics in pediatric CD. Infliximab is a chimeric monoclonal antibody against an important cytokine in the pro-inflammatory cascade, tumor necrosis factor alpha (TNF-$\alpha$). Infliximab has been shown to induce and maintain clinical remission in approximately 70% of patients with moderate to severe CD [3].

Multiple concurrent medications are often used in the treatment of CD. The use of an immunomodulator in conjunction with a biologic is called dual therapy. Historically, biologics were added to the treatment regimen only after failure of anti-inflammatory medications or immunomodulators, in what has been termed the “step-up” strategy of treating CD. However, given their efficacy, biologics are now being used more often as initial, first-line therapies, to induce and maintain remission in pediatric CD. This is called the “top-down” strategy [4].

There are several known side effects to biologic use, including anaphylaxis, hypersensitivity reactions, and increased susceptibility to infections. However, the long-term effects of the biologics are less known. There have been reports of increased rates of lymphoma among patients being treated with a biologic. This has also been seen in patients taking immunomodulators. The rates of skin cancers are also increased in patients taking immunomodulators. A particularly aggressive type of lymphoma, hepatosplenic T-cell lymphoma, has been reported in patients on dual therapy [5-6].

The question of when to stop biologic therapy in CD patients with long-standing deep clinical remission is important because biologic therapy is expensive and has potential long-term side effects. However, the data to support a de-escalation strategy is limited. Previous studies have mainly investigated stopping biologic therapy in adult patients receiving dual therapy [7]. Nonetheless, the results of these studies provide useful information that can be applied to formulating a de-escalation strategy for patients on monotherapy. A prospective

study investigating 115 CD patients in clinical remission on dual therapy found that after stopping infliximab, approximately half of the patients relapsed within 2 years. However, upon further analysis, the authors identified several laboratory parameters that significantly decreased the risk of relapse, including normal hemoglobin, C-reactive protein (CRP), and stool calprotectin levels. Amongst patients in the deepest remission, the risk of relapse decreased to 20%. Moreover, approximately 90% of the patients who did relapse were able to reestablish clinical remission with reintroduction of infliximab [8]. Most recently, Molander et al. looked at adult patients with inflammatory bowel disease in deep clinical and endoscopic remission being treated with biologics. Of the 52 total patients, 17 patients had a diagnosis of CD. Of these 17 patients, only 4 were on monotherapy with a biologic. At 1 year, 12/17 (70%) continued to be in clinical remission [9].

A recent study has investigated the question of withdrawing immunomodulator monotherapy in adult patients with CD in clinical remission. Kennedy et al. studied 237 patients with inflammatory bowel disease, including 129 patients with CD, for 12 months following withdrawal of immunomodulator monotherapy. At 12 months, 77% of patients continued to be in clinical remission. Normal CRP was found to significantly associated with decreased risk of relapse [10].

Therefore, the above studies show that within a subset of CD patients in deep clinical remission, de-escalation of biologic therapy or immunomodulator monotherapy may be effective.

Dietary therapy provides an alternative therapeutic modality for CD and may allow for de-escalation from medications. The exact pathophysiology of CD has not been fully elucidated, but genetics, environment, and the immune system each play a role in disease development and progression. In healthy individuals, the intestinal mucosa is regulated by a balance of pro-inflammatory and anti-inflammatory cytokines, but this equilibrium is disrupted in CD leading to tissue destruction. The microbiota, or the bacteria that naturally live in the human gastrointestinal tract, play a major role in CD [11]. A sequence of events involving an imbalance of protective and harmful bacteria (termed dysbiosis), defects in innate immunity, and subsequent adherence or translocation of bacteria may lead to an adaptive immune response that causes tissue injury and ulceration. This is termed the bacterial penetration cycle [12-13]. Dietary interventions may exert their effect by exclusion of dietary components that may generate dysbiosis, affect the mucous layer, increase intestinal permeability and/or allow adherence or translocation of harmful bacteria, such as adherent-invasive *E Coli* or diffusely-adherent *E Coli*.

There is strong evidence that providing complete nutrition with polymeric formula can induce and maintain clinical remission in up to 80% of patients with CD [14]. Although this treatment has no known side effects, it can impair quality of life as the diet is restrictive (only formula can be consumed) and is administered through a nasogastric tube. Therefore, studies have investigated the efficacy of liberalized dietary therapies. A diet providing 80-90% of calories from formula, with the remainder of the calories coming from a regular diet, was found to be effective for induction of remission in CD [15].

Dietary therapy may also be effective, if not underutilized, as maintenance therapy, but is fraught with obstacles. Longer term therapy with partial enteral nutrition (PEN) involving

50% of calories applied by nasogastric tube along with a low fat diet was shown to reduce relapse and reduce endoscopic recurrence compared with patients on a free diet [16-17]. PEN was also found to be as effective 6-mercaptopurine in another study [18]. However, long-term use of PEN, particularly if administered via feeding tube, may not be feasible for large segments of the population over prolonged periods of time**.** While sustained use of an elemental or polymeric formula is difficult, it is unclear if the formula itself provides a protective effect or rather the use of formula simply reduces the amount harmful foods consumed in a free diet. A recent study compared two groups of CD patients on enteral nutrition and showed that the group with higher intake of table foods had worse clinical outcomes [19]. This suggests that enteral nutrition is successful due to the restriction of harmful components of the diet rather than due to a protective effect of formula.

The findings from Sigall-Boneh et al. also support that it is diet rather than formula that may be most effective. Although subjects in that study did take 50% of their calories from formula, there were 7 patients who refused formula but still adhered to CDED**.** Of these, 6 still achieved remission with the CDED, alone, suggesting that the diet itself, rather than the formula, accounted for the clinical remission seen in the study [2].

**Compliance Statement**

This study will be conducted in full accordance all applicable Children's Hospital of Philadelphia Research Policies and Procedures and all applicable Federal and state laws and regulations including 45 CFR 46, and the HIPAA Privacy Rule. All episodes of noncompliance will be documented.

The investigators will perform the study in accordance with this protocol, will obtain consent and assent, and will report unanticipated problems involving risks to subjects or others in accordance with The Children's Hospital of Philadelphia IRB Policies and Procedures and all federal requirements. Collection, recording, and reporting of data will be accurate and will ensure the privacy, health, and welfare of research subjects during and after the study.

## 2 STUDY OBJECTIVES

The purpose of this pilot study is to determine clinical outcomes in a subset of pediatric CD patients in long-standing deep clinical remission following withdrawal of biologic or immunomodulator monotherapy based on the clinical judgment of their primary gastroenterologist and placement on either the CDED or an unrestricted diet.

### 2.1 Primary Objective (or Aim)

- To determine differences in time-to-relapse and the proportion of subjects with sustained relapse-free remission between subjects on the CDED and those on an unrestricted diet.

### 2.2 Secondary Objectives (or Aim)

The secondary objectives are to:

- To determine the proportion of all subjects with sustained relapse-free remission 52 weeks after withdrawal of biological or immunomodulator monotherapy (as part of clinical care)

- To assess the compliance of patients to the CDED over 52 weeks.

# 3 INVESTIGATIONAL PLAN

## 3.1 General Schema of Study Design

This will be an open-label, non-randomized pilot study involving 25 pediatric CD patients in deep clinical remission, who will be withdrawing from biologic or immunomodulator monotherapy based on the clinical judgment of their primary gastroenterologist. The study team will educate participants about the CDED. Participants who choose not to follow the diet will serve as controls. The study will include clinical and laboratory measures of disease activity obtained from chart review. Dietary compliance data will be collected prospectively. The study will be performed at one site – The Children's Hospital of Philadelphia. However, the research will also include outcome data from collaborators at Wolfson Medical Center in Israel running an independent study on medication withdrawal and the CDED in Crohn disease patients in deep remission. This data will come from individuals whose identity the investigators cannot readily ascertain, as the data will be de-identified. Additionally, data from protocols #15-011817 and #09-007286 will be used for data analysis for comparison. Data from these individuals will not be included in the enrollment numbers for this study and research activities using this will not be reported as part of the continuing review.

The cohort will be followed for 104 weeks (52 weeks of study participation with an additional 52 weeks of chart review).

### 3.1.1 Screening Phase

Potential subjects will be screened using the protocol inclusion and exclusion criteria. Parental/guardian permission (informed consent) and, if applicable, child assent, will be obtained prior to any study related procedures being performed.

### 3.1.2 Study Treatment Phase

Subjects who choose to follow the diet will attend a mandatory teaching session to learn more about the CDED. Subjects who do not wish to follow the CDED will serve as controls and will not attend the teaching session. During the treatment phase adherence to the diet will be determined based on 3-day food diaries, 24-hour dietary recalls, and questionnaires. Clinical response will be determined based stool samples for measurement of stool calprotectin and by calculation of Pediatric Crohn's Disease Activity Index (PCDAI) scores from medical record. Subjects will be followed for 52 weeks.

### 3.1.3 Follow-up Phase

The follow-up phase will occur during the subsequent 52 weeks and will consist of extraction of data from the medical chart.

### 3.2 Allocation to Treatment Groups and Blinding

This is an open-label, non-randomized, study. Therefore, this study is not blinded. Allocation to the treatment group (CDED) versus the control will be determined by whether the subject wishes to adhere to the CDED. Subjects who do not wish to adhere to the diet will serve as controls.

### 3.3 Study Duration, Enrollment and Number of Sites

#### 3.3.1 Duration of Study Participation

The study duration per subject will be a maximum of 104 weeks from enrollment. It is standard-of-care for CD patients withdrawing from therapy to follow-up in clinic every 6-8 weeks for the first year after withdrawal. The study will involve a baseline visit and follow-ups at outpatient GI visits during the first 52 weeks after withdrawal of therapy. For the subsequent 52 weeks, data will be collected from chart review, only, and there will be no study visits. There will be open communication via telephone calls to assess compliance and to answer any follow-up questions regarding the diet. A CHOP email address will also be provided to allow participants to ask follow-up questions regarding the diet and for participants to submit 3-day food diaries. In-person study visits will last approximately 10 minutes and will occur during regularly-scheduled GI appointments.

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

The study will be performed at The Children's Hospital of Philadelphia. The research will also include de-identified outcome data and samples from collaborators at Wolfson Medical Center in Israel running an independent study on medication withdrawal and the CDED in Crohn disease patients in deep remission. The data and samples from Wolfson Medical Center will come from individuals whose identity the investigators cannot readily ascertain, as the data will be de-identified. Data and samples from these individuals will not be included in the enrollment numbers for this study and research activities using this will not be reported as part of the continuing review. Additionally, data from protocols #15-011817 and #09-007286 will be used for data analysis for comparison.

The cohort will be followed for 104 weeks. Recruitment will stop when approximately 35 subjects are enrolled. Our goal is 25 evaluable subjects, of whom 10 will participate in the dietary intervention. We anticipate that recruitment will last 2 years from study initiation.

### 3.4 Study Population

#### 3.4.1 Inclusion Criteria

1) Males or females ages 10 to 21 years old with a diagnosis of CD using the Revised Porto criteria who will be withdrawing from biologic or immunomodulator monotherapy as part of clinical care.

2) Normal Growth Velocity, or Tanner 5

3) Steroid-free Remission (PCDAI <10 without the height component) for at least 6 months prior to enrollment
4) Colonoscopy during the preceding 3 months with complete mucosal healing or only few apthous ulcerations located in one segment
5) Stool calprotectin <250µg/g during the preceding 3 months
6) Parental/guardian permission (informed consent) and, if appropriate, child assent.

### 3.4.2 Exclusion Criteria

1) Discontinuation of biologic or immunomodulator therapy by the subject without the approval of the primary gastroenterologist.
2) Those subjects who in the judgment of the investigative team are unable to complete the study endpoints.

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

## 4 STUDY PROCEDURES

### 4.1 Screening Visit

Subjects being withdrawn from immunomodulator or biologic therapy will be approached to participate in this study once it is determined that they meet inclusion criteria. We will ascertain these subjects through a number of methods including electronic medical record query of patients in clinical remission and through GI physician referral. Subjects will be approached before or after a CHOP clinic visit or contacted by phone.

Those subjects who will not be at CHOP prior to the screening visit, a verbal screening consent form will be obtained before collecting any screening information. The screening consent will allow us to review their medical record to confirm eligibility and allow the subject to bring in a stool sample to the first visit. When consent is obtained initially by telephone, the person obtaining consent will document the participant's agreement to participate on the verbal consent form. All participants must provide written consent, and assent when appropriate, before undergoing any other study procedures. The study will be explained to each participant and their legal guardian when they come to the study site. If the subject and/or guardian agrees to participate, they will sign the written consent form. After informed consent is obtained, the patient will be enrolled.

The following will also be performed at the screening visit:

- Medical record review for extraction of clinical and laboratory data to allow for calculation of PCDAI
- 24 hour dietary recall for baseline diet information
- Stool, as well as oral and rectal swabs for microbiome analysis
- Information will be provided about the study diet. Subjects who choose to participate in the diet will be invited to a mandatory teaching session to learn

about the CDED. The teaching will be led by a study dietician and will involve detailed instruction regarding the use of the CDED and how to complete a food diary. These sessions will be held 1-2 times per month based on demand.

## 4.2 Study Treatment Phase

At the end of the teaching session subjects will identify a start date for the diet which will fall within 1 week of the teaching session.

### 4.2.1 Telephone Call (Week 1 +/- 3 days), Subjects on CDED only

- General questions to assess tolerability of CDED
- Modified MARS questionnaire to assess diet compliance
- 24 hour dietary recall
- Answer any follow-up questions about the diet

### 4.2.2 Email Communication (Week 3 +/- 3 days)

- 3-day Food Diary submitted via email
- If subjects are non-compliant with the diet at Week 3 then they will be moved to the control group.

### 4.2.3 Study Visit 1 (Week 6 +/- 10 days)

- Medical record review for extraction of clinical and laboratory data to allow for calculation of PCDAI
- Submission of stool samples for measurements of stool calprotectin and microbiome
- Oral and rectal swabs for microbiome analysis
- Modified MARS questionnaire to assess diet compliance (subjects on CDED only)
- 24 hour dietary recall

### 4.2.4 Telephone Call (Week 7 +/- 3 days), Subjects on CDED only

- General questions to assess tolerability of CDED
- Modified MARS questionnaire to assess diet compliance
- Answer any follow-up questions about the diet

#### 4.2.5 Email Communication (Week 9 +/- 3 days)

- 3-day Food Diary submitted via email

#### 4.2.6 Study Visit 2 (Week 12 +/- 10 days)

- Medical record review for extraction of clinical and laboratory data to allow for calculation of PCDAI
- Submission of stool samples for measurements of stool calprotectin
- Modified MARS questionnaire to assess diet compliance (subjects on CDED only)
- 24 hour dietary recall

#### 4.2.7 Telephone Call (Week 13 +/- 3 days), (Subjects on CDED only)

- Questions to assess tolerability of CDED
- Modified MARS questionnaire to assess diet compliance
- Answer any follow-up questions about the diet

#### 4.2.8 Study Visit 3 (Week 24 +/- 10 days)

- Medical record review for extraction of clinical and laboratory data to allow for calculation of PCDAI
- Submission of stool samples for measurement of stool calprotectin
- Modified MARS questionnaire to assess diet compliance (subjects on CDED only)
- 3-day Food Diary (to be completed before appointment)

#### 4.2.9 Study Visit 4 (Week 36 +/- 10 days)

- Medical record review for extraction of clinical and laboratory data to allow for calculation of PCDAI
- Submission of stool samples for measurement of stool calprotectin
- Modified MARS questionnaire to assess diet compliance (subjects on CDED only)
- 24 hour dietary recall

#### 4.2.10 Study Visit 5 (Week 52)

- Medical record review for extraction of clinical and laboratory data to allow for calculation of PCDAI
- Submission of stool samples for measurements of stool calprotectin and microbiome
- Oral and rectal swabs for microbiome analysis
- Modified MARS questionnaire to assess diet compliance (subjects CDED only)
- 24 hour dietary recall

#### 4.2.11 Clinical Flare/Relapse Visit

- Clinical relapse will be determined by the primary gastroenterologist
- Modified MARS questionnaire to assess diet compliance (subjects CDED only)
- 24 hour dietary recall
- Submission of stool samples for measurement of stool calprotectin and microbiome
- Oral and rectal swabs for microbiome analysis

### 4.3 Medical record review after the first 52 weeks of therapy withdrawal

- Medical record review only
- The following data will be extracted from the medical record:

(1) Sustained relapse-free remission/time to relapse

(2) Concurrent medications

(3) Growth parameters, including height, weight, DEXA scans

(4) Endoscopy, imaging pathology reports, if applicable

(5) Surgery or hospitalizations after the first 52 weeks of therapy, if applicable

(6) Laboratory data, including hematocrit, serum albumin, C-reactive protein, sedimentation rate, and stool calprotectin

### 4.4 Unscheduled Visits

Subjects may have a clinical flare of Crohn disease (as determined by the primary gastroenterologist) during the study. If the subject is in the office for a clinical flare appointment, a 24 hour dietary recall, stool for calprotectin microbiome, oral and rectal swabs for microbiome analysis, as well as a modified MARS questionnaire will be performed, as well as a medical record review.

### 4.5 Subject Completion/Withdrawal

Subjects may withdraw from the study at any time without prejudice to their care. They may also be discontinued from the study at the discretion of the Investigator for lack of adherence to study treatment or visit schedules or AEs. The Investigator may also withdraw subjects who violate the study plan, or to protect the subject for reasons of safety or for administrative reasons. It will be documented whether or not each subject completes the clinical study. If the Investigator becomes aware of any serious, related adverse events after the subject completes or withdraws from the study, they will be recorded in the source documents.

#### 4.5.1 Early Termination Study Visit

Subjects who withdraw from the study will have all procedures enumerated for the last visit as the early termination visit.

### 4.6 Data Pooling

The study population for this study includes individuals who have been receiving ongoing care at The Children's Hospital of Philadelphia. Many subjects are currently, previously, or will be enrolled in other research studies within the Division of Gastroenterology. Some members of this study team also serve as study team members on the other studies.

Data and samples that have previously, or will be collected as part of participation in other studies may be helpful for the current protocol. By signing the consent form, subjects will grant access to their data and biospecimens from other studies. Doing so may avoid the necessity of repeating tests and procedures. This may minimize the risks to subjects and will reduce duplication. Subjects will only have their data and biospecimens from other studies used if they consent to sharing the results as part of this study.

## 5 STUDY EVALUATIONS AND MEASUREMENTS

### 5.1 Screening and Monitoring Evaluations and Measurements

#### 5.1.1 Medical Record Review

The following data and dates of service will be abstracted from the medical chart (paper or electronic) for all subjects.

- Date of birth

- NIH ethnicity
- Diagnosis
- Date of diagnosis
- Disease location
- Weight
- Height
- Body mass index
- Laboratory values
- History of previous abdominal and perianal surgeries
- List of current and historical medications
- Reports from any previous endoscopies or radiological tests, including pathology biopsy results
- Extraintestinal manifestations such as frank arthritis, primary sclerosing cholangitis, and/or uveitis
- PCDAI
- Environmental history
- Surgical History

### 5.1.2 Pediatric Crohn Disease Activity Index (PCDAI)

A PCDAI will be extracted from the medical chart at baseline and at every visit for every subject. The PCDAI is a validated measure of disease severity [20]. It includes subjective patient reporting of symptoms, physical examination, growth parameters, and several laboratory tests (hematocrit, erythrocyte sedimentation rate, and serum albumin). Scores range from 0 to 100. Scores of 0-10 correspond to no disease activity, 11 – 30 represents mild disease, and a score greater than 30 is defined as moderate to severe disease activity. It is standard-of-care for the PCDAI to be calculated at each office visit by the primary gastroenterologist, so this information will be obtained from chart review.

### 5.1.3 MARS Questionnaire

The modified MARS Questionnaire is a validated measure of medication adherence. It will be administered by a study member during each study visit, but only to subjects on the CDED.

### 5.1.4 3-day Food Diary

A 3-day food diary will be filled out by all subjects, including those on an unrestricted diet, prior to several study visits. At weeks 3 and 9 subjects will email the completed 3-day food diary to the CHOP IBD Research Center email address, accessible only to study team members. Subjects will receive detailed instructions regarding how to complete a food diary during the mandatory informational diet session. The food diary will document all food and drink intake, including estimated portion sizes, during a 3-day period, and will be helpful in determining caloric intake and adherence to the diet, if applicable. Food Processor Nutrition Analysis Software will be used to analyze the food diary. This program is currently used clinically within the Division of Gastroenterology and provides all the features needed to accurately analyze a 3-day food diary for the purposes of this study.

### 5.1.5 24 Hour Dietary Recall

A 24 hour dietary recall will be conducted by a study member during several study visits. All subjects, including those on an unrestricted diet, will recall their food/drink intake during the previous 24 hours. This will be helpful in determining caloric intake and adherence to the diet, if applicable. Food Processor Nutrition Analysis Software will be used to analyze the dietary recall. This program is currently used clinically within the Division of Gastroenterology and provides all the features needed to accurately analyze a 24 hour dietary recall for the purposes of this study. In general, recall of dietary intake can be very subjective and is prone to potential inaccuracies. By using two different methods of capturing dietary intake (the recall and the food diary) analyzed by experienced members of the study team (registered dieticians and gastroenterologists) the investigators will be able to accurately measure caloric intake in our participants.

If a subject fails to meet 100% of his/her estimated nutritional needs during analysis of a 24 hour dietary recall or a 3-day food diary (based on established World Health Organization or Recommended Daily Allowance guidelines), then the subject will be instructed to modify his/her intake within the confines of the diet, including the use of a nutritional supplement of the subject's choosing.

### 5.1.6 Stool

Stool for measurement of stool calprotectin will be collected and analyzed at the CHOP central lab. The results of each stool calprotectin will be entered into the electronic medical record. Stool calprotectin is used as a marker of intestinal inflammation. It is standard-of-care at the CHOP Inflammatory Bowel Center to consider any stool calprotectin value above 250µg/g as significantly elevated. If a study participant has a stool calprotectin above 250µg/g during the course of the study, the primary gastroenterologist will be notified of the lab value either in person or via email. The study will cover the costs of measuring the stool calprotectin, unless already being ordered for clinical care. All other laboratory data will be obtained from chart review only.

Additionally, some of the stool sample will be used to sample the luminal contents of the intestine. If the stool is collected at home prior to arrival at the study visit then the subject will store the stool in the refrigerator prior to submitting it to a study team member. Stool

samples will be returned to CHOP using pre-paid mailers, by dropping it off at the main hospital or a satellite location, or by submitting it to a study team member at a study visit. Samples will be refrigerated and then stored at -80˚C or in a liquid nitrogen freezer. For DNA isolation, DNA extraction kits will be used.

### 5.1.7 Rectal Swabs

Two rectal swab samples will be obtained by research staff or clinical team members. Each sterile swab will be inserted 2-3 cm into the rectum, turned 360 degrees, removed, placed into the original tubes and sealed tightly and stored frozen at -80°C or in a liquid nitrogen freezer until analysis. DNA will be purified from swabs in 96-well plates using the Mo-Bio PowerSoil® kits and utilizing Eppendorf epMotion® automated liquid handling.

### 5.1.8 Oral Swabs

Two oral swab samples will be obtained using sterile swabs. A study team member will instruct subjects on how to obtain oral swab samples. Samples will be stored at at -80°C or in a liquid nitrogen freezer until analysis. DNA will be purified from swabs in 96-well plates using the Mo-Bio PowerSoil® kits and utilizing Eppendorf epMotion® automated liquid handling.

### 5.1.9 Environmental Swab

At the time of the rectal or oral swab collection, an environmental control sample will be obtained from the room air. If subjects are collecting samples at home, they will be provided instructions for collecting the environmental swab.

## 5.2 Efficacy Evaluations

The efficacy of the study intervention will be determined by comparing the differences in time-to-relapse and the proportion of subjects with sustained relapse-free remission between subjects on the CDED and those who choose not to follow the CDED. Remission will be assessed using PCDAI, CRP, and stool calprotectin.

**Diagnostic Tests, Scales, Measures, etc.**

The results of each stool calprotectin will be entered into CHOP's electronic medical record and shared with the participant's primary gastroenterologist. Stool calprotectin is used as a marker of intestinal inflammation. It is standard-of-care at the CHOP Inflammatory Bowel Center to consider any stool calprotectin value above 250µg/g as significantly elevated. If a study participant has a stool calprotectin above 250µg/g during the course of the study, the primary gastroenterologist will be notified of the lab value either in person or via email. The modified MARS questionnaire, 3-day calorie count, and 24-hour dietary recall will be used to measure dietary adherence.

## 5.3 Safety Evaluation

All adverse events will be documented. All decisions related to restarting biological of immunomodulator therapy will be made by the primary gastroenterologist.

# 6 STATISTICAL CONSIDERATIONS

## 6.1 Primary Endpoint

The primary endpoint will be differences in time-to-relapse and sustained relapse-free remission at 52 weeks, as assessed using PCDAI, CRP, microbiome, and stool calprotectin, between subjects on the CDED and subjects on a free diet.

## 6.2 Secondary Endpoints

Secondary endpoints will include the following:

- Time-to-relapse and sustained relapse-free remission at 52 weeks, as assessed using PCDAI, CRP, microbiome, and stool calprotectin, in all subjects.
- Compliance with the dietary intervention as measured by the modified MARS questionnaire

## 6.3 Control of Bias and Confounding

Subjects being withdrawn from immunomodulator or biologic therapy will be approached to participate in this study. We will ascertain these subjects through a number of methods including query of the electronic medical record for patients in clinical remission and through GI physician referral. With these measures, we believe we will capture all subjects eligible and avoid bias.

## 6.4 Statistical Methods

### 6.4.1 Baseline Data

Standard descriptive statistics will be used to describe subject characteristics, which will include information such as the distribution of age, race/ethnicity, and disease characteristics. Summary statistics such as means, standard deviations, medians, and ranges will be compiled for all measured variables. Frequencies will be computed for all categorical and ordinal variables. Box plots will be used to examine distributions, identify potential influential points, and guide in the choice of transforming if warranted. Additionally, data from protocols #15-011817 and #09-007286 will be used for data analysis for comparison.

### 6.4.2 Analysis of Primary Outcomes of Interest

The primary analysis will include all subjects meeting all inclusion and exclusion criteria and completing the first study visit. The primary endpoint will be sustained relapse-free remission at 52 weeks and will be expressed as a percentage with a 95% confidence interval. Time-to-relapse analysis comparing free diet and the CDED will be performed using a Cox regression model.

### 6.5 Sample Size and Power

There is no pediatric literature on withdrawing immunomodulator or biological monotherapy in CD patients in deep clinical remission. Therefore, this pilot study is needed to determine effect size for a larger randomized controlled study. We have approximately 120 patients in clinical remission on biological monotherapy. This does not include patients on immunomodulator monotherapy. Unfortunately, the number of patients in remission who have already undergone immunomodulator/biologic withdrawal by their primary gastroenterologists at this center is not available. However, even if this data were available it would not truly reflect the feasibility of recruitment at the current moment as medication withdrawal has been receiving more attention within the pediatric gastroenterology community over the last several months. The target sample size will be to enroll 35 subjects to yield 25 eligible subjects, 10 of whom would participate in the CDED, which is a reasonable number of patients to recruit over the two years of expected recruitment and should provide sufficient data regarding an effect size for future, larger, studies. This study will also be helpful in determining how many subjects will elect to adhere to the CDED versus staying on the regular diet.

## 7 SAFETY MANAGEMENT

### 7.1 Clinical Adverse Events

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

### 7.2 Adverse Event Reporting

The majority of this study is observational in nature. The dietary intervention is not expected to pose a risk greater than minimal risk. Therefore, SAEs are not expected. If any unanticipated problems related to the research involving risks to subjects or others happen during the course of this study (including SAEs) these will be reported to the IRB in accordance with CHOP IRB SOP 408: Unanticipated Problems Involving Risks to Subjects. AEs that are not serious but that are notable and could involve risks to subjects will be summarized in narrative or other format and submitted to the IRB at the time of continuing review.

## 8 STUDY ADMINISTRATION

### 8.1 Data Collection and Management

1. Confidentiality of Data. In order to ensure confidentiality, each subject will be given a study ID number**.** There will be a master list saved on a secure CHOP password-protected drive that will link the personal health information used to identify the patient with their study ID number. Data will be entered into a password protected REDCap database and maintained on a secure CHOP server. The study team will collect the information and will be responsible for data management and accuracy of records. Only the study team on this protocol will have access to the data. Data and backups will be stored in the CHOP Research Information Systems Storage Area Network (SAN). Access to the SAN directories where data are stored will be limited to study personnel, with authentication performed using CHOP's enterprise Active

Directory service. All information collected in this study will be kept confidential as required by law.

**2.** Security/Recovery of Data. The password-protected REDCap database will be maintained on a secure CHOP server, and it will also be saved on a secure CHOP password-protected drive.

3. In addition to REDCap, the study will use an electronic honest broker developed and managed by CHOP's Department of Biomedical and Health Informatics (DBHi) (f.k.a. Center for Biomedical Informatics).

   The Electronic Honest Broker (eHB): The eHB is a software service that interconnects research systems and the research portal (described below) by escrowing and securely storing Protected Health Information (PHI) and the variable associations with research systems. The eHB encrypts this information on the client side before storing this sensitive information. Persons or organizations who are named as the actual keepers of the honest broker can call the eHB Application Programming Interface (API) to re-identify and supplement subject data and/or specimens for predefined reasons. Research staff on the study can request re-identification of subject records from the eHB by written request to the DBHi for quality assurance and quality control purposes only.

   Subject information will be entered into the Biorepository Portal (BRP) and stored in the eHB encrypted database. These PHI elements include:

   - Medical Record Number or Other Unique Identifier
   - Last Name
   - First Name
   - Date Of Birth

   Holding these identifiers in the eHB allows for creation of a unique subject list that can be linked to research data and specimen records on demand as data is entered. The BRP is a federated web application used to support longitudinal biospecimen collection activities. Using HTTP protocols, it interfaces with downstream data systems, including REDCap and Thermo Scientific's Nautilus LIMS, to provide a single unified interface for data collection and update activities. Data backup is performed nightly via a dedicated backup system. The backup environment is maintained by a dedicated staff using dedicated resources. Access to the backup environment is restricted to Research Information Systems staff.

### 8.1.1 Data sources

Data will be collected from the medical records at CHOP (EPIC, ChartMaxx, paper records, etc). If the subject is not a CHOP patient, we will request the subject to sign an Authorization to Release/Obtain Patient Information form so we can obtain the medical records from the primary gastroenterologist. Additionally, using the honest broker clinical

information can be pulled from the clinical data warehouse, surescripts, and clarity. Data will be entered into a CHOP REDCap database and the BRP that will only be accessible to members of the research team. The research will also include de-identified data and samples from a collaborator (Dr. Arie Levine) at the Wolfson Medical Center in Israel running a study with a similar protocol. And coded data from IRB #15-011817 and #09-007286 obtained through an honest broker. The data and samples will come from individuals whose identity the investigators cannot readily ascertain, as the data will be de-identified to the CEASE study team. Data and samples from these individuals will not be included in the enrollment numbers for this study and research activities using this will not be reported as part of the continuing review, but may be included in data analysis. The data for these protocols is stored within CHOP's secure research drives. There is limited access to these drives with required approval to gain access. If data is shared outside of CHOP, the send secure CHOP email will be utilized.

## 8.2 Confidentiality

All data and records generated during this study will be kept confidential in accordance with Institutional policies and HIPAA on subject privacy. The investigators and other site personnel will not use such data and records for any purpose other than conducting the study. Safeguards to maintain subject confidentiality are described under Data Collection and Management – Confidentiality. No identifiable data will be used for future study without first obtaining IRB approval. The investigators and other site personnel will not use such data and records for any purpose other than conducting the study, and the protected health information collected will not be reused or disclosed to any other person or entity, except as required by law or for authorized oversight of the research project. The following groups of people at CHOP may have access to this information: the study team and the Hospital's Institutional Review Board and compliance office.

## 8.3 Regulatory and Ethical Considerations

### 8.3.1 Data and Safety Monitoring Plan

The safety and monitoring plan for this project will focus on safeguards to ensure confidentiality as described in Section 8.1. Unanticipated problems involving risks to subjects will be monitored over the course of the study by the Principal Investigator.

### 8.3.2 Risk Assessment

The only additional procedures will be collection of stool, rectal swabs, and oral swabs. Additionally, subjects will have the option to receive the dietary intervention. Therefore, participation in this study is minimal risk. Collecting stool may contain germs that spread disease. Subjects will be reminded to carefully wash their hands and use careful handling techniques to avoid spreading infection. Subjects may experience small physical discomfort while having the swab samples taken. However, once the swab is removed, the discomfort will be alleviated.

The intervention diet restricts potentially unhealthy components of diet, such dairy, wheat products, sauces, soft drinks, fruit juice, and processed meats. Therefore, the diet may restrict foods that the patient may enjoy and may limit necessary nutrients. Routine detailed

dietary recall recorded by study members (who are registered dieticians or physicians) at study visits will assess the caloric and nutrient intake of the subjects' diet. If a subject does not meet 100% of estimated nutritional needs (based on standard World Health Organization equations for resting energy expenditure or Recommended Daily Allowance guidelines) when assessed during any 24 hour dietary recall or 3-day calorie count then study members may suggest modification of a subject's intake within the confines of the diet, including the use of a nutritional supplement of the subject's choosing. If at the time of the next visit the subject continues to not meet at least 75% his/her caloric needs then the subject will be removed from the dietary intervention.

While in this study a subject may have a Crohn disease flare. This will be determined by the primary gastroenterologist. The decision to take subjects off of Crohn disease medications will be made by the primary gastroenterologist and any decision to go back on medication will also be made by the primary gastroenterologist, as part of clinical care. Healthier diets have never been shown to induce a Crohn flare. Therefore participation in this study should not put subjects at increased risk for a flare.

### 8.3.3 Potential Benefits of Study Participation

The direct benefit to a subject's participation in this study may include improved clinical outcomes after withdrawal of medical therapy while on the intervention diet. For patients who do not choose to participate in the dietary intervention there are no direct benefits. There are, however, positive, indirect benefits to society to be gained from this study since the study will allow for a better understanding of de-escalation strategies in pediatric patients with Crohn disease.

### 8.3.4 Risk-Benefit Assessment

The potential benefits of the patients who participate in the dietary intervention outweigh any potential risks in this study, as the diet may potentially increase their chances of staying in remission. For patients not involved in the diet there are no direct individual benefits. However, the risks of participating in this study are minimal, and these risks are outweighed by the benefits to society to be gained from new knowledge about de-escalation strategies in pediatric patients with CD.

## 8.4 Recruitment Strategy

Subjects being withdrawn from immunomodulator or biologic therapy will be approached to participate in this study. We will ascertain these subjects through a number of methods including query of the electronic medical record for patients in clinical remission and through GI physician referral. Study team members will make contact with potential subjects to describe the study and enroll patients who are interested in participating. No advertising will be used. We expect to enroll sufficient number of subjects to achieve our study aims.

## 8.5 Informed Consent/Assent and HIPAA Authorization

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

The study will be thoroughly explained by study personnel, including the study rationale and goals. Potential participants will be given the opportunity to ask questions about the study, risks, benefits, and confidentiality. A member of the research team will explain each section of the consent form and questions will be encouraged. Written consent will be obtained after all the questions and discussions have been completed. For the subjects under 18 years of age, assent will be obtained by explaining briefly the study while the parent/guardian is present. Assent will be documented in writing on the consent form. Participation in all areas of the study will be completely voluntary. Should the subjects become intolerant of any aspect of the study, their participation will be discontinued. Subjects who turn 18 during the course of the study will be re-consented.

### 8.5.1 Waiver of Documentation of Consent

For potential subjects who are not at CHOP prior to enrollment, they may be contacted by phone. A verbal screening informed consent form will be obtained before collecting any screening information. If there are questions regarding eligibility, relevant information from the patient's medical record will be sought from their treating physician. In addition, subjects may be asked to provide a stool sample before coming to the study site. When consent is obtained initially by telephone, the person obtaining consent will document the participant's agreement to participate on the verbal screening informed consent form. All participants must provide written consent, and assent when appropriate, before undergoing any other study procedures.

### 8.5.2 Waiver of Assent

A full waiver of assent is requested only for the verbal screening consent form which can be done over the telephone with parents/guardian and children may not be available. At the enrollment visit, the study will be reviewed in its entirety with the participant and the participant's parents/guardian. Written assent will be obtained at this in-person visit and documented on the main consent form

### 8.5.3 Waiver of HIPAA Authorization

A partial waiver of HIPAA authorization to obtain verbal authorization is requested for subjects enrolled who will need to provide research samples prior to being seen at CHOP.

## 8.6 Payment to Subjects/Families

Subjects will receive a $10 prepaid bankcard each time rectal and oral swabs are obtained (up to 3 times during the 1-year study period) and each time a stool sample is completed (up to 6 times during the study period). The maximum amount that a subject will receive during this study is $90 in gift cards. The cost of measuring the stool calprotectin will be covered by the investigators and the families will not be charged. All other lab measures will be ordered by the primary gastroenterologist per standard of care.

## 9 PUBLICATION

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

## 10 REFERENCES


1. Rabizadeh S, Dubinsky M. Update in pediatric inflammatory bowel disease. *Rheum Dis Clin North Am*. 2013 Nov;39(4):789-99.

2. Sigall-Boneh R, Pfeffer-Gik T, Segal I, Zangen T, Boaz M, Levine A. Partial enteral nutrition with a crohn's disease exclusion diet is effective for induction of remission in children and young adults with crohn's disease. *Inflamm Bowel Dis*. 2014 Aug;20(8):1353-60.

3. Hyams J, Crandall W, Kugathasan S, Griffiths A, Olson A, Johanns J, et al. Induction and maintenance infliximab therapy for the treatment of moderate-to-severe crohn's disease in children. *Gastroenterology*. 2007 Mar;132(3):863,73; quiz 1165-6.

4. Antunes O, Filippi J, Hebuterne X, Peyrin-Biroulet L. Treatment algorithms in crohn's - up, down or something else? *Best Pract Res Clin Gastroenterol*. 2014 Jun;28(3):473-83.

5. Brown SL, Greene MH, Gershon SK, Edwards ET, Braun MM. Tumor necrosis factor antagonist therapy and lymphoma development: Twenty-six cases reported to the food and drug administration. *Arthritis Rheum*. 2002 Dec;46(12):3151-8.

6. Cucchiara S, Escher JC, Hildebrand H, Amil-Dias J, Stronati L, Ruemmele FM. Pediatric inflammatory bowel diseases and the risk of lymphoma: Should we revise our treatment strategies? *J Pediatr Gastroenterol Nutr*. 2009 Mar;48(3):257-67.

7. Pariente B, Laharie D. Review article: Why, when and how to de-escalate therapy in inflammatory bowel diseases. *Aliment Pharmacol Ther*. 2014 Aug;40(4):338-53.

8. Louis E, Mary JY, Vernier-Massouille G, Grimaud JC, Bouhnik Y, Laharie D, et al. Maintenance of remission among patients with crohn's disease on antimetabolite therapy after infliximab therapy is stopped. *Gastroenterology*. 2012 Jan;142(1):63,70.e5; quiz e31.

9. Molander P, Farkkila M, Salminen K, Kemppainen H, Blomster T, Koskela R, Jussila A, Rautiainen H, Nissinen M, Haapamaki J, Arkkila P, Nieminen U, Kuisma J, Punkkinen J, Kolho K, Mustonen H, Sipponen T. Outcome after discontinuation of TNF-α-blocking therapy in patients with inflammatory bowel disease in deep remission. *Inflamm Bowel Dis.* 2014; 20:1021-1028.

10. Kennedy NA, Kalla R, Warner B, Gambles CJ, Musy R, Reynolds S, Dattani R, Nayee H, Felwick R, Harris R, Marriott S, Senanayake SM, Lamb CA, Al-Hilou H, Gaya DR, Irving PM, Mansfield J, Parkes M, Ahmad T, Cummings JRF, Amott ID, Satsangi J, Lobo AJ, Smith M, Lindsay JO, Lees CW. Thiopurine withdrawal using sustained

clinical response in inflammatory bowel disease: relapse and recapture rates, with predictive factors in 237 patients. *Aliment Pharmacol Ther*. 2014; 40: 1313-1323.

11. Scaldaferri F, Gerardi V, Lopetuso LR, Del Zompo F, Mangiola F, Boskoski I, et al. Gut microbial flora, prebiotics, and probiotics in IBD: Their current usage and utility. *Biomed Res Int*. 2013;2013:435268.

12. Levine A, Wine E. Effects of enteral nutrition on Crohn disease: Clues to the impact of diet on disease pathogenesis**.** *Inflamm Bowel Dis* 2013; 19:1322-9

13. Pfeffer Gik T, Levine A. Dietary Clues to Pathogenesis of Crohn's disease. *Dig Dis* 2014;32:389-394.

14. Fell JM, Paintin M, Arnaud-Battandier F, Beattie RM, Hollis A, Kitching P, et al. Mucosal healing and a fall in mucosal pro-inflammatory cytokine mRNA induced by a specific oral polymeric diet in paediatric crohn's disease. *Aliment Pharmacol Ther*. 2000 Mar;14(3):281-9

15. Gupta K, Noble A, Kachelries KE, Albenberg L, Kelsen JR, Grossman AB, et al. A novel enteral nutrition protocol for the treatment of pediatric crohn's disease. *Inflamm Bowel Dis*. 2013 Jun;19(7):1374-8.

16. Yamamoto T, Nakahigashi M, Saniabadi AR, Iwata T, Maruyama Y, Umegae S, Matsumoto K. Impacts of long-term enteral nutrition on clinical and endoscopic disease activities and mucosal cytokines during remission in patients with Crohn's disease: a prospective study. *Inflamm Bowel Dis*. 2007;13:1493-501

17. Yamamoto T, Nakahigashi M, Umegae S, Kitagawa T, Matsumoto K. Impact of long-term enteral nutrition on clinical and endoscopic recurrence after resection for Crohn's disease: A prospective, non-randomized, parallel, controlled study. *Aliment Pharmacol Ther.* 2007;1;25:67-72

18. Hanai H, Iida T, Takeuchi K, Arai H, Arai O, Abe J, Tanaka T, Maruyama Y, Ikeya K, Sugimoto K, Nakamura T, Nakamura K, Watanabe F. Nutritional therapy versus 6-mercaptopurine as maintenance therapy in patients with Crohn's disease. *Dig Liver Dis*. 2012;44:649-54

19. Lee D, Baldassano RN, Compher C, Albenberg L, Otley A, Griffiths A, Bushman F, Wu GD, Lewis JD.: *Effectiveness of nutritional and anti-TNF therapy in North American children with active Crohn's disease. Poster presentation*. North American Society for Pediatric Gastroenterology, Hepatology and Nutrition (NASPGHAN) 2014 Annual Meeting, Atlanta GA; JPGN. 59((S2)): S37, Oct 23-26 2014.

20. Hyams, J.S., et al., Development and validation of a pediatric Crohn's disease activity index. *J Pediatr Gastroenterol Nutr*, 1991. **12**(4): p. 439-47.