Personalized AZithromycin/metronidAZole, in combination with standard induction therapy, to achieve a fecal microbiome community structure and metagenome changes associated with sustained remission in pediatric Crohn's Disease (CD): a pilot study

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Personalized AZithromycin/metronidAZole, in combination with standard induction therapy, to achieve a fecal microbiome community structure and metagenome changes associated with sustained remission in pediatric Crohn's Disease (CD): a pilot study

The PAZAZ Study

Protocol Number: 140041

National Clinical Trial (NCT) Identified Number: NCT04186247

Sponsor Investigator: Johan Van Limbergen, MD, FRCPCH, PhD

Monitor: Alimentiv B.V. (formerly Robarts Clinical Trials Inc.)

Funded by: Crohn's and Colitis Foundation of America

Version Number: v.1.9

October 12, 2021

Summary of Changes from Previous Version:

Version Date	Affected Section(s)	Summary of Revisions Made	Rationale
1.9 October 2021	6.3	Use CastorEDC randomization function instead of an additional separate program (SPSS)	To use only one program (CastorEDC) as eCRF and randomization tool to minimize mistakes
1.8 March 2021	4.1	Adding study timepoint window of +/- 5 days in case needed	To facilitate planning of study visits
1.8 March 2021	6.3, 10.1.3, 10.1.4	Changed IWK health center to Amsterdam UMC, location AMC as principal receiver of trial data and biological samples	PI moved to Amsterdam and trial will be coordinated from Amsterdam UMC, location AMC
1.8 March 2021	1.1	Updated participating sites	North Carolina Children's Hospital (US), Sheba Medical Centre (Israel), Location VUMC of Amsterdam UMC (The Netherlands) will no longer be participating.
1.8 March 2021	10.1.5	Changed medical monitor to Prof. Anthony Otley	Prof. Antony Otley (local PI IWK Canada) will be the new medical monitor as former medical monitor prof. Francisco Sylvester (UNC) will no longer be involved in the study.
1.8 March 2021	10.1.9.1	Changed electronic data capture system to Castor	Castor EDC is license is provided by the Amsterdam UMC, the coordinating center of this trial.
1.8		Updated trial monitor name to Alimentiv B.V.	Alimentiv B.V. is the new name of the PAZAZ trial monitor, formerly known as Robarts Clinical Trial Inc.

October 2020	Addendum for the Netherlands	Feasibility study clarification	Required by National Ethics Review Board in the Netherlands October 2020
May 2021	Addendum for the Netherlands	Specifying chosen medicinal products for the AmsterdamUMC.	Metronidazole 500 mg, Metronidazole 250 mg, Azitromycin 250 mg will be the medicinal products used in the AmsterdamUMC.

Table of Contents

STATEMENT OF COMPLIANCE

The trial will be conducted in accordance with International Conference on Harmonisation Good Clinical Practice (ICH GCP), applicable United States (US) Code of Federal Regulations (CFR), and Crohn's & Colitis Foundation Terms and Conditions of Award. The Principal Investigator will assure that no deviation from, or changes to the protocol will take place without prior agreement from the Investigational New Drug (IND) or Investigational Device Exemption (IDE) sponsor, funding agency and documented approval from the Institutional Review Board (IRB), except where necessary to eliminate an immediate hazard(s) to the trial participants. All personnel involved in the conduct of this study have completed Human Subjects Protection and ICH GCP Training.

The protocol, informed consent form(s), recruitment materials, and all participant materials will be submitted to the IRB for review and approval. Approval of both the protocol and the consent form must be obtained before any participant is enrolled. Any amendment to the protocol will require review and approval by the IRB before the changes are implemented to the study. All changes to the consent form will be IRB approved; a determination will be made regarding whether a new consent needs to be obtained from participants who provided consent, using a previously approved consent form.

1 PROTOCOL SUMMARY

1.1 SYNOPSIS

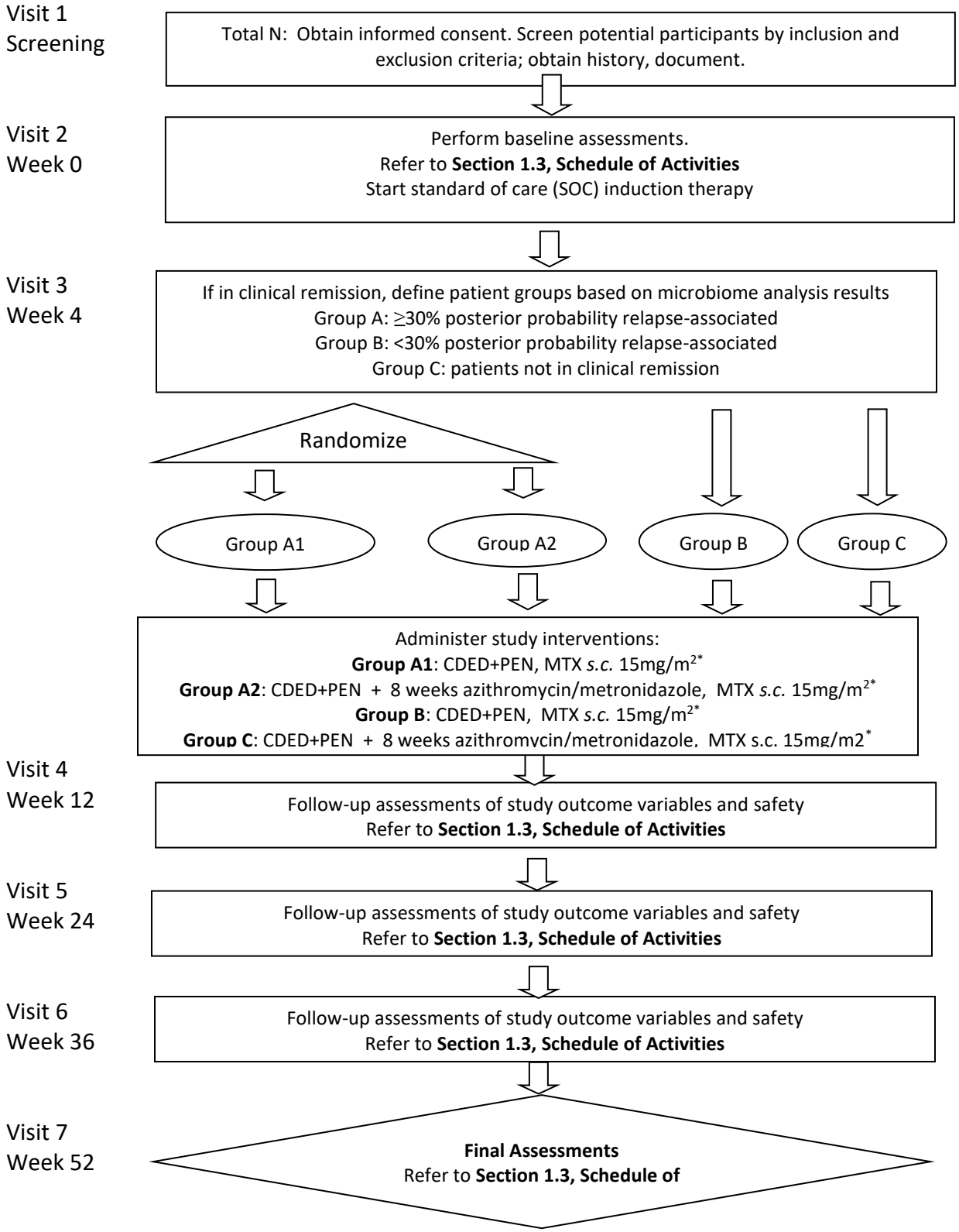
Title:	PAZAZ: Personalized azithromycin/metronidazole, in combination with standard induction therapy, to achieve a fecal microbiome community structure and metagenome changes associated with sustained remission in pediatric Crohn's Disease (CD)-- a pilot study
Study Description:	This is a multi-center, randomized, controlled open-label add-on design trial pilot study to evaluate the efficacy of personalized adjunctive antibiotic (azithromycin + metronidazole) therapy in pediatric subjects with mild to moderate Crohn's disease (CD) who have a relapse-associated microbiome profile. The study hypothesis is that adjunctive antibiotic therapy will improve clinical response to standard of care (SOC) induction therapy in a subgroup of CD patients with a relapse-associated microbiome profile. This is an add-on design trial for subjects already receiving SOC induction therapy; there will be no placebos. Prior to starting SOC induction therapy at week 0, subjects will provide a baseline stool sample that will be screened for microbiome profiles associated with risk of relapse according to an established statistical model. At week 4, subjects with a relapse-associated microbiome will be randomized into either a control arm that will continue to receive SOC induction therapy for an additional 8 weeks, or a treatment arm that will receive adjunctive antibiotic therapy in addition to continuing to receive SOC induction therapy for an additional 8 weeks. Subjects who do not have a relapse-associated microbiome will enter a separate control arm that will continue to receive SOC induction therapy and will have data collected for exploratory objectives. Subjects who are not in clinical

	remission by week 4 will receive antibiotic therapy regardless of microbiome signature at baseline. Subjects will be monitored for an additional 40 weeks after the treatment period (52 weeks total).
Objectives:	Primary Objective: To evaluate the potential efficacy of personalized adjunctive antibiotic therapy in maintaining remission in pediatric subjects undergoing SOC induction therapy for mild to moderate Crohn's disease. Secondary Objective: To evaluate longitudinal (clustered and with respect to baseline) changes in disease activity indices (PCDAI) components and inflammatory markers in stool and blood. Exploratory Objectives: To investigate relationship between changes in subject microbiome composition and changes in disease activity over time.
Outcome variables:	Primary Outcome variable: Sustained remission defined as no need of re-induction for clinical flare (new course of nutritional therapy, need to start steroids), steroid dependence, biologic (anti-TNF) use, and/or intestinal surgery by 12 months. Secondary Outcome variables: Longitudinal (clustered and with respect to baseline) changes in disease activity indices (PCDAI: 0-100) components and inflammatory markers in stool and blood at each study visit up to 12 months. Longitudinal (clustered and with respect to baseline) changes in patient-reported outcomes (PRO) by 12 months. Exploratory Outcome variable: Longitudinal (clustered and with respect to baseline) changes in fecal microbiome taxonomic composition or in total gene (metagenome) content. The changes will be analyzed for association with changes in disease activity (e.g. relapse or sustained remission) over time up to 12 months.
Study Population:	Children aged 3 to 17 years (inclusive) with mild to moderately active Crohn's disease who will receive standard of care induction therapy.
Phase:	2
Description of Sites/Facilities Enrolling Participants:	This is a multi-center trial with eight participating sites in Canada, the U.S.A. the Netherlands, and Israel. The sites are pediatric gastroenterology clinics located at: 1. Dalhousie University, IWK Health Centre, Halifax, Nova Scotia (Canada) 2. University of California San Francisco, Benioff Children's Hospital, San Francisco, CA 3. University of Pittsburgh Medical Center, Children's Hospital of Pittsburgh, Pittsburgh, PA 4. Amsterdam University Medical Centers, Academic Medical Center. Amsterdam, The Netherlands

5. Wolfson Medical Centre, University of Tel Aviv, Tel Aviv, Israel

Description of Study Intervention:	Antibiotics will be administered orally for an 8-week period. Azithromycin will be administered at a dose of 7.5mg/kg to a maximum of 500mg/day for 5 consecutive days per week for the first 4 weeks and then 3 consecutive days/week for 4 weeks. Metronidazole will be administered 10mg/kg twice daily to a maximum of 1000mg/day for 8 weeks.
Study Duration:	3 years
Participant Duration:	The duration of study participation is 52 weeks (12 months)

1.2 SCHEMA



*see text 4.1 for guidance

1.3 SCHEDULE OF ACTIVITIES (SOA)

Procedures	Visit 1 Screening ≤30 days prior to week 0	Visit2 Week 0	Study Visit3 Week 4	Study Visit 4 Week 12	Study Visit 5 Week 24	Study Visit6 Week 36	Final Study Visit 7 Week 52
Informed consent	X						
Demographic data	X						
Medical history	X	X	X	X	X	X	X
Review inclusion/exclusion criteria	X	X					
Concomitant medication review	X	X	X	X	X	X	X
Physical exam (including height and weight)	X	X	X	X	X	X	X
Medication calendar distribution and teaching		X					
Blood sample [a]	X	X[b]	X	X	X	X	X
Calculate PCDAI score (0-100)	X	X[b]	X	X	X	X	X
Stool sample (16S rRNA)	X	X[b]	X	X	X	X	X
Stool sample (FCP)	X	X[b]	X	X	X	X	X
Stool sample (C. difficile infection testing) [c]	X						
Urine pregnancy test [d]	X		X				
Adverse event review and evaluation			X	X	X	X	X
Randomization			X				
ECG, if applicable [e]			X				
Administer antibiotic, if applicable			X				
Teaching for antibiotic medication calendar, if applicable			X				
Complete Case Report Forms (CRFs)	X	X	X	X	X	X	X
IMPACT (ages 9 and older)		X		X	X		X

A window of study timepoint +/- 5 days is allowed for all visits/questionnaires/sampling.
a: Includes hematology and clinical chemistry Albumin, C reactive protein (CRP), hematocrit (HCT), erythrocyte sedimentation rate (ESR), alanine transaminase (ALT), aspartate transaminase (AST), creatinine, and bilirubin. Total blood volume collected at each visit will be 5ml.
b: The timing of the Screening and Week 0 visits will depend on the start of therapy. Week 0 blood and stool samples will not be repeated if provided during the screening visit, provided that the stool samples were collected prior to bowel prep.
C: If performed for clinical indication, as per standard of care
d: Only applicable for post-menarchal female participants initiating methotrexate

Procedures	Visit 1 Screening ≤30 days prior to week 0	Visit2 Week 0	Study Visit3 Week 4	Study Visit 4 Week 12	Study Visit 5 Week 24	Study Visit6 Week 36	Final Study Visit 7 Week 52
e: All patients randomized to group A2 and C will receive an ECG exam both prior to and at 2-4 weeks after the initiation of the study drug							

2 INTRODUCTION

2.1 STUDY RATIONALE

Crohn's disease (CD) is a major phenotype of inflammatory bowel disease (IBD) characterized by transmural (often granulomatous and patchy) inflammation throughout the gastrointestinal (GI) tract, most often involving the ileum and colon. Chronic inflammation within the GI tract can cause progressive tissue damage leading to serious complications requiring surgery, including intra-abdominal abscesses, fistulas, and intestinal strictures.[1] CD is highly heterogeneous with respect to age of onset, disease location (i.e. anatomical extent) and behavior (i.e. inflammatory or stricturing/penetrating disease). Disease course is also highly variable among patients with some experiencing chronically active severe disease while other have intermittent periods of clinical remission and disease exacerbation (i.e. 'flares'). Patient responses to therapy are also highly variable, but the reasons for this are not fully understood.

Current treatments for CD aim to not only control symptoms but to maintain clinical remission and mucosal healing (deep remission).[2] Corticosteroids, enteral nutrition, thiopurines, methotrexate and biologics (such as monoclonal antibodies against tumour necrosis factor-α: 'anti-TNF') are effective to modulate inflammatory activity, but surgery is still frequently required.[3]

The exact cause of CD is unclear, but current thinking holds that disease results from a defective or inappropriate activation of the mucosal immune system response to commensal gut microbiota, collectively termed the gut microbiome.[1] Certain bacteria can adhere to and invade epithelial cells of the inflamed mucosa and granulomas to replicate inside macrophage phagolysosomes.[4] Numerous CD susceptibility genes are involved in pathways that govern innate immunity, recognition of bacterial pathogens, and handling of intracellular bacteria.[5] Diversion of the fecal stream can lead to clinical improvement in medically refractory Crohn's colitis.[6]

The field of microbiome research has grown exponentially over the past several years, catalyzed by the US-led Human Microbiome Project (HMP)[7] and the European-led MetaHIT[8] project. Studies using next-generation sequencing and new bioinformatics approaches have begun to characterize fundamental differences in the gut microbiome in children (and adults) with CD versus healthy controls. [9-11] The ileum and colon is densely populated with a variety of metabolically active bacteria that interact with the host immune system. The collective genomic content of the microbiome—the metagenome—has been

estimated to contain at least 100-times more genes than the human genome. The influence of the microbiome on disease pathogenesis and progression in CD is a significant area of research interest, since a breakdown in the balance between protective and harmful bacteria (termed 'dysbiosis') is the current prevailing hypothesis for the development of CD.[12] Animal studies have shown that bacterial load and the composition of bacterial communities can influence both the site and degree of inflammation in the GI tract.[13,14] Several studies applying microbial profiling and in-depth sequencing techniques to the collective DNA content of gut microbiota (i.e. microbiome) of CD patients have shown distinct microbiome profiles associated with different clinical outcomes or responses to treatment.[3,15-19]

The probable role for bacteria in triggering disease activity implies that antibiotics could have a role in CD therapy. However, while several meta-analyses support the use of antibiotics in controlling luminal inflammation, results of individual trials are heterogeneous.[2,20] Recently, a combination of azithromycin/metronidazole has been found to be superior to metronidazole alone for induction of remission and improvement in fecal calprotectin. [21]However, individual responses to this antibiotic treatment were variable, showing the percentage of clinical response to equal that of remission. This "all or nothing" phenomenon suggests that the effect of antibiotics may depend on the type of microbiota involved and their susceptibility to antibiotics. Antibiotics used in previous trials such as rifaximin are non-absorbable and would be less effective against bacteria that have already translocated into tissue.[21-23]

Recently, the Crohn's Disease Exclusion diet was shown to be associated with comparable efficacy but superior tolerance and maintenance of remission than exclusive enteral nutrition in a randomized controlled trial.[24]

The purpose for the proposed pilot trial is to determine whether personalized, microbiome-informed administration of an azithromycin/metronidazole antibiotic can improve clinical response to nutritional induction therapy and prolong remission compared to induction therapy alone.

2.2 BACKGROUND

Current role of antibiotics in pediatric Crohn's disease treatment
The most recently reported consensus guidelines of European Crohn's and Colitis Organization (ECCO)/European Society of Pediatric gastroenterology, Hepatology and Nutrition (ESPGHAN) on the medical management of pediatric Crohn's disease in 2014 include the following on antibiotics:

> "Antibiotics, such as metronidazole or ciprofloxacin, are recommended in the treatment of perianal fistulizing disease (EL 3 (pediatrics) EL1 (adults)) 80% agreement…In more severe perianal fistulizing disease, antibiotics should be used as adjuvant (EL3)) 88% agreement
>
> Practice points: 1. In perianal disease, metronidazole/ciprofloxacin-based treatments have a good short-term response and may offer a bridge to immunosuppressive medications;
> 2. Usual daily doses for metronidazole are 10–20 mg/kg, and for ciprofloxacin 20 mg/kg;
> 3. Azithromycin and rifaximin may be useful for induction of remission in children with mild to moderate luminal inflammatory pediatric CD; 4. There is no evidence to recommend the use of anti-mycobacterial antibiotics."[2]

Antibiotics such as metronidazole or ciproflaxin are regularly used in CD standard care to treat penetrating complications such as perianal fistulizing disease or abdominal abscesses in children and adults.[2] Multiple trials have shown that metronidazole at a dosage of 10-20mg/kg/day may improve or heal perianal CD and the drug is now widely used for this indication in children and adults with CD.[2] The most recent guidelines published by the World Gastroenterology Organization support the use of antibiotics in perianal disease, fistulizing, and bacterial overgrowth secondary to stricturing disease.[25] There is also extensive evidence regarding antibiotic use in post-operative CD management.[25] Long-term metronidazole use (20mg/kg/day for 3 months) has been shown to prevent recurrence of Crohn's after ileal resection in adults with CD.[26]

Potential role for antibiotics in Crohn's disease treatment

The probable role of bacteria in triggering disease activity in CD has led to the use of antibiotic therapy in mild to moderate luminal disease but previous trials have produced mixed results.[27,28] [29] In a recent systematic review including 10 randomized controlled trials (RCT) (1160 patients), there was a statistically significant effect of antibiotics being superior to placebo. Different antibiotics were administered (anti-tuberculosis therapy, macrolides, fluroquinolones, 5-nitroimidazoles, and rifaximin) either alone or in combination.[29] Antibiotics are proposed to work by reducing bacterial load, limiting bacterial translocation, and reducing the concentration of adherent bacteria in the lumen and mucosa.[25] Ideally, antibiotics used to treat CD should be active in the lumen, biofilms, and inside host cells in order to target adherent and invasive bacteria thought to provoke disease. Unlike quinolones, rifaximin or azoles, azithromycin (a macrolide antibiotic) has excellent tissue penetration, is able to maintain relatively high luminal concentrations and is effective against biofilms.[30,31]

Macrolide antibiotics are also known to have anti-inflammatory and immune modulating effects and have been explored in several clinical trials for their effects in patients with chronic lung diseases.[32,33] A recent multicentre, double-blind, randomized, parallel-group, placebo-controlled trial (ACTRN12610000383066) in children aged 1-8 with either non-cystic-fibrosis bronchiectasis or chronic suppurative lung disease found that once-weekly azithromycin for up to 24 months decreased pulmonary exacerbations.[34] The dose used was 30mg/kg per week and was well-tolerated with no serious adverse events being attributed to the intervention. However, antibiotic use was also accompanied by increased carriage of azithromycin-resistant bacteria, of which the clinical consequences are uncertain.[34]

Azithromycin + metronidazole-based therapy for inducing remission in pediatric Crohn's disease (CD)

Levine and Turner conducted a retrospective analysis of 32 children with active CD who were treated with an 8 week course of combined azithromycin and metronidazole identical to the dosage proposed in this trial: azithromycin was given 7.5–10 mg/kg, once daily (maximal dose: 500 mg), for five consecutive days/ week for 4 weeks, and 3 times a week for the following 4 weeks, in conjunction with metronidazole. Azithromycin-based therapy was applied due to its effect in inducing apoptosis (down regulation of anti-apoptotic transcription factor Bcl-xL), efficacy against biofilms and intracellular bacteria. Clinical remission was observed in 21/32 (66%) patients and 54% of children with elevated CRP at baseline normalized their C-reactive protein (CRP).[35]

More recently, Levine et al. performed a randomized controlled trial (NCT01596894) allocating children with mild to moderately active disease into groups receiving azithromycin + metronidazole therapy (7.5mg/kg azithromycin once daily for five consecutive days/ week for 4 weeks, and 3 times a week for the following 4 weeks, in conjunction with 20mg/kg/day of metronidazole) or metronidazole alone daily for 8 weeks.[21] The results of this trial show that remission rates with azithromycin + metronidazole

therapy are better than with metronidazole alone, with a 66% clinical remission. There were no drug-related serious adverse events (SAEs) in the study.

Personalized adjunctive antibiotic therapy in pediatric Crohn's disease treatment
We have recently shown that the use of immunomodulators and the need for treatment escalation to anti-TNF or surgery are comparable after 6 years of follow-up, following induction of remission using either exclusive enteral nutrition (EEN) or steroids, both of which are used as 'standard-of-care' induction therapy in pediatric CD.[36] We also showed that a baseline (B) fecal microbiome signature with predominance of Proteobacteria, does not correct with nutritional therapy by week 12 and is associated with recurrence of disease.[37] We have recently shown, in an independent cohort of CD patients, that remission due to nutritional therapy corrects features of the dysbiosis described in the study by Gevers et al. in the RISK-CCFA cohort of treatment naïve pediatric CD.[38]

We have recently shown that the Crohn's Disease Exclusion Diet (CDED) + Partial Enteral Nutrition (PEN) has similar efficacy to EEN but has significantly better tolerance and sustained remission. Dietary induced remission with CDED+PEN by 12 weeks resulted in sustained improvement in Proteobacteria.

We hypothesize that the risk of early flare after achieving clinical remission using standard-of-care nutritional induction therapy in CD patients with a relapse-associated (i.e. Proteobacteria-rich) microbiome signature can be reduced via personalized adjunctive induction therapy with antibiotics (azithromycin + metronidazole).

2.3 RISK/BENEFIT ASSESSMENT

2.3.1 KNOWN POTENTIAL RISKS

Drug Risks:
Azithromycin[39] (see Attachments for complete product package insert)
In clinical trials, most of the reported side effects were mild to moderate in severity and were reversible upon discontinuation of the drug. Potentially serious side effects of angioedema and cholestatic jaundice were reported rarely. Most of the side effects leading to discontinuation were related to the gastrointestinal tract, e.g., nausea, vomiting, diarrhea, or abdominal pain.

In adult studies, side effects that occurred with a frequency of 1% or less included the following:

Cardiovascular: Palpitations, chest pain.
Gastrointestinal: Dyspepsia, flatulence, vomiting, melena and cholestatic jaundice.
Genitourinary: Monilia, vaginitis and nephritis.
Nervous System: Dizziness, headache, vertigo and somnolence.
General: Fatigue.
Allergic: Rash, pruritus, photosensitivity and angioedema.

The side effects in pediatric patients are comparable to those seen in adults, with different incidence rates for the dosage regimens recommended in pediatric patients. In pediatric studies, side effects that occurred with a frequency of 1% or less included the following:

Cardiovascular: Chest pain.
Gastrointestinal: Dyspepsia, constipation, anorexia, enteritis, flatulence, gastritis, jaundice, loose stools and oral moniliasis.
Hematologic and Lymphatic: Anemia and leukopenia.
Nervous System: Headache (otitis media dosage), hyperkinesia, dizziness, agitation, nervousness and insomnia.
General: Fever, face edema, fatigue, fungal infection, malaise and pain.
Allergic: Rash and allergic reaction.
Respiratory: Cough increased, pharyngitis, pleural effusion and rhinitis.
Skin and Appendages: Eczema, fungal dermatitis, pruritus, sweating, urticaria and vesiculobullous rash.
Special Senses: Conjunctivitis

Metronidazole[40] (see Attachments for complete product package insert)
The following reactions have been reported during treatment with metronidazole:

Central Nervous System: The most serious adverse reactions reported in patients treated with metronidazole have been convulsive seizures, encephalopathy, aseptic meningitis, optic and peripheral neuropathy, the latter characterized mainly by numbness or paresthesia of an extremity. Since persistent peripheral neuropathy has been reported in some patients receiving prolonged administration of metronidazole, patients should be specifically warned about these reactions and should be told to stop the drug and report immediately to their physicians if any neurologic symptoms occur. In addition, patients have reported headache, syncope, dizziness, vertigo, incoordination, ataxia, confusion, dysarthria, irritability, depression, weakness, and insomnia (see WARNINGS).
Gastrointestinal: The most common adverse reactions reported have been referable to the gastrointestinal tract, particularly nausea, sometimes accompanied by headache, anorexia, and occasionally vomiting; diarrhea; epigastric distress; abdominal cramping; and constipation.

Mouth: A sharp, unpleasant metallic taste is not unusual. Furry tongue, glossitis, and stomatitis have occurred; these may be associated with a sudden overgrowth of Candida which may occur during therapy.

Dermatologic: Erythematous rash and pruritus.

Hematopoietic: Reversible neutropenia (leukopenia); rarely, reversible thrombocytopenia.

Cardiovascular: Flattening of the T-wave may be seen in electrocardiographic tracings.

Hypersensitivity: Urticaria, erythematous rash, Stevens-Johnson Syndrome, toxic epidermal necrolysis, flushing, nasal congestion, dryness of the mouth (or vagina or vulva), and fever.

Renal: Dysuria, cystitis, polyuria, incontinence, and a sense of pelvic pressure. Instances of darkened urine have been reported by approximately one patient in 100,000. Although the pigment which is probably responsible for this phenomenon has not been positively identified, it is almost certainly a metabolite of metronidazole and seems to have no clinical significance.

Other: Proliferation of Candida in the vagina, dyspareunia, decrease of libido, proctitis, and fleeting joint pains sometimes resembling “serum sickness.” Rare cases of pancreatitis, which generally abated on withdrawal of the drug, have been reported.

Patients with CD are known to have an increased incidence of gastrointestinal and certain extra-intestinal cancers. There have been some reports in the medical literature of breast and colon cancer in Crohn’s disease patients who have been treated with metronidazole at high doses for extended periods of time. A cause and effect relationship has not been established.

Methotrexate:
The most frequently reported adverse reactions include ulcerative stomatitis, leukopenia, nausea, and abdominal distress. Other frequently reported adverse effects are malaise, undue fatigue, chills and fever, dizziness and decreased resistance to infection.

Other adverse reactions that have been reported with methotrexate are listed below by organ system.
Alimentary System: gingivitis, pharyngitis, stomatitis, anorexia, nausea, vomiting, diarrhea, hematemesis, melena, gastrointestinal ulceration and bleeding, enteritis, pancreatitis.

Blood and Lymphatic System Disorders: suppressed hematopoiesis, anemia, aplastic anemia, pancytopenia, leukopenia, neutropenia, thrombocytopenia, agranulocytosis, eosinophilia, lymphadenopathy and lymphoproliferative disorders (including reversible). Hypogammaglobulinemia has been reported rarely.

Cardiovascular: pericarditis, pericardial effusion, hypotension, and thromboembolic events (including arterial thrombosis, cerebral thrombosis, deep vein thrombosis, retinal vein thrombosis, thrombophlebitis, and pulmonary embolus).

Central Nervous System: headaches, drowsiness, blurred vision, transient blindness, speech impairment including dysarthria and aphasia, hemiparesis, paresis and convulsions have also occurred following administration of methotrexate. Following low doses, there have been occasional reports of transient subtle cognitive dysfunction, mood alteration or unusual cranial sensations, leukoencephalopathy, or encephalopathy.

Hepatobiliary Disorders: hepatotoxicity, acute hepatitis, chronic fibrosis and cirrhosis, hepatic failure, decrease in serum albumin, liver enzyme elevations.

Infection: There have been case reports of sometimes fatal opportunistic infections in patients receiving methotrexate therapy for neoplastic and non-neoplastic diseases. Pneumocystis carinii pneumonia was the most common opportunistic infection. There have also been reports of infections, pneumonia, Cytomegalovirus infection, including cytomegaloviral pneumonia, sepsis, fatal sepsis, nocardiosis; histoplasmosis, cryptococcosis, Herpes zoster, H. simplex hepatitis, and disseminated H. simplex.

Musculoskeletal System: stress fracture.

Ophthalmic: conjunctivitis, serious visual changes of unknown etiology.

Pulmonary System: respiratory fibrosis, respiratory failure, alveolitis, interstitial pneumonitis deaths have been reported, and chronic interstitial obstructive pulmonary disease has occasionally occurred.

Skin: erythematous rashes, pruritus, urticaria, photosensitivity, pigmentary changes, alopecia, ecchymosis, telangiectasia, acne, furunculosis, erythema multiforme, toxic epidermal necrolysis, Stevens-Johnson syndrome, skin necrosis, skin ulceration and exfoliative dermatitis
Urogenital System: severe nephropathy or renal failure, azotemia, cystitis, hematuria, proteinuria; defective oogenesis or spermatogenesis, transient oligospermia, menstrual dysfunction, vaginal discharge, and gynecomastia; infertility, abortion, fetal death, fetal defects.

Other rarer reactions related to or attributed to the use of methotrexate such as nodulosis, vasculitis, arthralgia/myalgia, loss of libido/impotence, diabetes, osteoporosis, sudden death, lymphoma, including reversible lymphomas, tumor lysis syndrome, soft tissue necrosis and osteonecrosis. Anaphylactoid reactions have been reported.

Blood Draws: Phlebotomy can cause discomfort, bruising, a small risk of infection, or thrombosis. Standard aseptic technique will be employed to prevent infection. All blood draws will take place at the time of routine blood test monitoring. The volume of blood required to perform the clinical tests described in this study is 5 ml. A total of 30 ml is expected to be collected over the 52-week study period. These volumes are well below the recommended blood draw volume limits for pediatric study participants—5 ml/kg in a single day and 9.5 ml/kg over an 8 week period—as described by NIH guidelines.

Stool Sample: There is no specific risk to the subject in obtaining a stool sample though bringing a stool sample from home may be inconvenient.

Breach of confidentiality: There is a risk that someone not authorized to view participant information, including identifiable information, will gain access to this information. Clinical sites will take measures to prevent this from happening and will comply with local regulations.

2.3.2 KNOWN POTENTIAL BENEFITS

A retrospective uncontrolled analysis of pediatric subjects with active CD showed that combined azithromycin-metronidazole therapy may be effective in inducing remission.[35] A recent randomized controlled trial (NCT01596894) allocating children with mild to moderately active CD into groups receiving azithromycin + metronidazole therapy (7.5mg/kg azithromycin once daily for five consecutive days/ week for 4 weeks, and 3 times a week for the following 4 weeks, in conjunction with 20mg/kg/day of metronidazole) or metronidazole alone daily for 8 weeks, showed that remission rates with azithromycin + metronidazole therapy are better than with metronidazole alone, with a 66% clinical remission. [21]

This study may not result in any direct benefit to participants. However, results of this study could help determine whether personalized adjunct antibiotic therapy can improve the efficacy of current standard treatment protocols for children and adolescents with CD.

2.3.3 ASSESSMENT OF POTENTIAL RISKS AND BENEFITS

The available information suggests that the present clinical study has an acceptable risk-benefit ratio.

Approximately 50% of pediatric patients with CD will relapse within a year of starting induction therapy and require repeated courses of induction therapy and/or treatment escalation.[41,42] Subjects in the antibiotic treatment arm of this trial will continue to receive standard therapy and are at comparable risk of relapse as subjects in the control arms. In group C of subjects not achieving remission with nutritional induction therapy alone, there will be no randomization and antibiotics will be given as additional induction treatment. The patient, parents and treating physician are free at all times to choose any treatment outside of the study.

The risk of rare, but serious, heart-related side effects from the antibiotic drug azithromycin are minimized by screening patients for heart rhythm irregularities prior to administering antibiotics. Metronidazole presents minimal risk to the subjects and is routinely used in this pediatric population. The safety monitoring practices employed by this protocol are adequate to protect the subjects' safety and detect all anticipated adverse events.

Subjects in the antibiotic intervention arm may experience direct health benefit through the study treatment by reducing their risk of disease flare thus avoiding additional intestinal damage as well as additional immune suppression or surgery. There will be no additional direct benefit for subjects in the reference arms because they will be receiving standard care. The knowledge gained from this trial could lead to improved treatment approaches for a subgroup of patients who have a relapse-associated microbiome profile.

3 OBJECTIVES AND OUTCOME VARIABLES

OBJECTIVES	OUTCOME VARIABLES	JUSTIFICATION FOR OUTCOME VARIABLES
Primary		
To evaluate the potential efficacy of personalized adjunctive antibiotic therapy in maintaining clinical remission in pediatric subjects undergoing SOC induction therapy for mild to moderate Crohn's disease who have a relapse-associated microbiome profile	Proportion of subjects in sustained remission at 52 weeks after starting standard of care induction therapy Sustained remission is defined PCDAI less than or equal to 10 AND no need of re-induction for clinical flare (new course of EEN, need to restart steroids), no steroid dependence, no biologic (anti-TNF) use, and no intestinal surgery by 12 months.	We will aim to demonstrate superiority of adjunctive antibiotic therapy + SOC over SOC alone in patients with a relapse-associated microbiome. Group A1 and A2 will be compared and sustained remission is the principal analysis variable.
Secondary		

OBJECTIVES	OUTCOME VARIABLES	JUSTIFICATION FOR OUTCOME VARIABLES
To evaluate the potential efficacy of personalized adjunctive antibiotic therapy in improving PRO, components of established disease activity measures in remission, as well as 'biochemical' remission in pediatric subjects undergoing SOC induction therapy for mild to moderate Crohn's disease who have a relapse-associated microbiome profile	Longitudinal (clustered and with respect to baseline) changes in disease activity indices (PCDAI) components, and inflammatory markers in blood and stool: • PCDAI score (0-100) • Normalization of CRP (mg/dL) • Fecal calprotectin (FCP) (microgram/g) Longitudinal (clustered and with respect to baseline) changes PRO: • IMPACT (self-report, ages 9-17 years)	Multiple secondary variables are needed to describe clinically relevant treatment benefits. Depending on the results of the microbiome analysis, either groups A2 and B will be pooled and compared with group A1 or groups A1 and B will be pooled and compared with group A2 (section 9.4.3).
Tertiary/Exploratory		
To investigate relationship between changes in subject microbiome composition and changes in disease activity over time.	Longitudinal (clustered and with respect to baseline) changes in fecal microbiome taxonomic composition or in total gene (metagenome) content. The changes will be analyzed for association with changes in disease activity (e.g. relapse or sustained remission) over time.	Not applicable; these changes are exploratory and not intended to demonstrate clinical benefit of treatment

4 STUDY DESIGN

4.1 OVERALL DESIGN

The primary hypothesis tested is that administering adjunctive antibiotic therapy to patients with a relapse-associated gut microbiome profile will help to sustain clinical response to standard induction therapy for mild to moderately active Crohn's disease.

To test this hypothesis, patients with a relapse-associated microbiome profile will be identified via baseline stool sample analysis and will be randomly allocated to the treatment arm that will receive antibiotics (azithromycin + metronidazole) on top of SOC induction therapy, or a control arm that will receive SOC induction therapy alone. Patients not achieving clinical remission (PCDAI greater than 10 at week 4), can receive antibiotics (azithromycin/metronidazole), will not be randomized, and will continue to be followed until 52 weeks. This pilot trial is intended to be a smaller version of a larger trial, and will be used to evaluate feasibility including standard deviation of the outcome measures, adherence, and baseline microbiome profiling.

Standard of care (SOC) induction therapy, as started by the responsible physician, for this study will be defined as Crohn's Disease Exclusion diet nutritional therapy (CDED Phase 1 from 0-6 weeks and Phase 2 from 6-12 weeks), in accordance with the recently published RCT for the CDED.[24]

This is a 52-week, multicenter, randomized, controlled, open-label add-on design clinical trial pilot trial to test the feasibility of a larger trial evaluating the efficacy of adjuvant antibiotic therapy in pediatric patients with mild to moderate CD. This study will not be blinded.

A total of 20 subjects between the ages of 3 and 17 years inclusive will be enrolled in eight investigational sites located in Canada, the United States, the Netherlands and Israel.

Subjects will be stratified based on microbiome profile before they are randomized. A baseline stool sample provided at or within 30 days of week 0 will be processed for DNA sequencing and analyzed by a blinded bioinformatician at Dalhousie University. At week 4, subjects will be assigned to Group A or B based on posterior probability of having a 'relapse-associated' microbiome according to an established statistical model. If patients have not achieved clinical remission, they will be assigned to group C to receive antibiotic for 8 weeks. Subjects in Group A will be randomly assigned to continue SOC therapy alone (Group A1) or to receive add-on antibiotic therapy in addition to SOC (Group A2) for the next 8 weeks (week 4-12). Subjects in Group B will continue on SOC therapy alone for the next 8 weeks.

At week 0, a medication calendar will be distributed to all subjects and patients/families will receive teaching on how to complete it for SOC therapy. At week 4, subjects randomized to Group A2 will be given additional instructions on how to complete the calendar for antibiotic treatment. The calendar will be returned to study staff at week 12 to be used as a measure of compliance and adherence.

Pediatric patients with mild to moderate CD are normally started on an immunomodulator during induction therapy to help maintain remission. Although methotrexate is not FDA approved for this purpose, in this study, subjects will receive 15mg/m^2 subcutaneous (s.c.) methotrexate (MTX) as maintenance therapy (max. 25mg weekly), consistent with several current international guidelines as standard of care[46], unless there are contraindications, or oral MTX is preferred and/or the patient/family refuses immunomodulatory therapy.[47] However, the use of MTX is not a condition to take part in this study. The use of MTX is only prescribed under guidance of the treating physician.
Because methotrexate depletes folic acid, patients receiving methotrexate may also receive folic acid supplementation under the guidance of the treating gastroenterologist. Reflecting the variability of maintenance options in current clinical care [21], the recommendation of MTX s.c. as maintenance therapy is intended to help mitigate the confounding effect of different types or lack of immunomodulatory therapy but is not intended to trigger patients being excluded from or leaving the study, e.g. if a treating physician prefers to continue azathioprine, then MTX will not be started concurrently. Details of immunomodulatory therapy will be recorded on a study CRF for concomitant medications.

Participants will complete the following PRO questionnaire at week 0, 12, 24 and 52 study visits:

1. IMPACT (self-report, ages 9-17 years):
   https://mirapeds.ca/miraresearch/impact/

If needed, a study timepoint window of +/- 5 days is allowed for all visits/questionnaires/sampling.

4.2 SCIENTIFIC RATIONALE FOR STUDY DESIGN

The experimental treatment arm (Group A2) will receive an add-on therapy given on top of existing SOC induction therapy and the control groups will receive SOC induction therapy alone. This will be a superiority study to demonstrate that add-on antibiotic therapy is superior to existing standard of care.

The control arms (Group A1 and B) are stratified based on microbiome profile. This will allow us to confirm our previous observations that subjects with a relapse-associated microbiome profile are at an increased risk of relapse after completing induction therapy. The primary objective will be to compare the rates of sustained remission between Groups A1 and A2 to determine whether add-on antibiotic therapy improves outcomes for patients with a relapse-associated microbiome profile. Group C will allow patients to receive additional antibiotic for a separate indication of induction of remission, as this group has not received clinical remission on nutritional therapy alone by week 4.

4.3 JUSTIFICATION FOR DOSE

Azithromycin and metronidazole will be administered orally as a tablet as according to their FDA-approved labels, or as a suspension if required (see Attachments). Both azithromycin and metronidazole are available in 250mg tablets, doses will be given in multiple of 125mg (1/2 tablets).

Azithromycin will be administered at a dose of 7.5mg/kg to a maximum of 500mg/day for 5 consecutive days per week for the first 4 weeks and then 3 consecutive days/week for 4 weeks. Metronidazole will be administered 10mg/kg twice daily to a maximum of 1000mg/day for 8 weeks. There will be no placebos administered in this study. These dosages are based on the recent RCT of azithromycin + metronidazole.[21]

4.4 END OF STUDY DEFINITION

A participant is considered to have completed the study if he or she has completed all phases of the study including the last visit or the last scheduled procedure shown in the Schedule of Activities (SoA), Section 1.3.

The end of the study is defined as completion of the last visit or procedure shown in the SoA in the trial globally.

5 STUDY POPULATION

5.1 INCLUSION CRITERIA

In order to be eligible to participate in this study, an individual must meet all of the following criteria:

1. Provision of signed and dated informed consent form (and assent form, as applicable)
2. Stated willingness to comply with all study procedures and availability for the duration of the study
3. Male or female, aged 3 to 17 years
4. Diagnosed with CD according to standard clinical and histological criteria, within 36 months of week 0

5. Exhibiting mild to moderate symptoms of active disease, as determined by a PCDAI score >10 (or >7.5 excluding the height item) and ≤37.5
6. Evidence of active inflammation based on either: fecal calprotectin level >=250 microgram/g (local laboratory or pre-arranged sponsor testing) within 30 days prior to week 0 visit; or according to accepted endoscopic and histologic evidence obtained during an endoscopy procedure completed within 30 days prior to Week 0 Visit.

5.2 EXCLUSION CRITERIA

An individual who meets any of the following criteria will be excluded from participation in this study:

1. Current or previous use of anti-TNF or other biologic therapy
2. Presence of stricturing, penetrating (intestinal or perianal) and/or fistulizing CD.
3. Pregnancy or lactation
4. Have undergone intestinal resection
5. Laboratory diagnosis of Clostridium Difficile Infection (CDI), if performed for clinical indication
6. Treatment with another investigational drug or other intervention within 30 days before week 0
7. Risk factors for arrhythmia including history of prolonged QTc, hypokalemia or hypomagnesemia, resting bradycardia, or concurrent treatment with other drugs with potential for QT prolongation.
8. History of Cockayne syndrome
9. Prior diagnosis of any hematologic condition/blood dyscrasia which may result in leukopenia (even if leukocyte count is normal at screening)
10. Known allergy or intolerance to azithromycin or metronidazole
11. Subjects who received IV anti-infective within 35 days prior to week 0 visit or oral anti-infectives within 14 days prior to the week 0 visit.
12. Subject on oral aminosalicylates who has not been on stable doses for greater than, or discontinued within, at least 14 days prior to week 0.
13. Subject on cyclosporine, tacrolimus or mycophenolate mofetil. Stable doses (no change within 14 days prior to week 0) of Azathioprine, 6-mercaptopurine or MTX are not a reason for exclusion.
14. Subject who received fecal microbial transplantation within 35 days prior to week 0 visit.
15. Screening laboratory and other analyses show any of the following abnormal results:
    - AST, ALT > 2 X upper limit of the reference range (as determined locally at each site)
    - Urea, Creatinine > 1.5X upper limit of the reference range (as determined locally at each site)
    - White blood cell (WBC) count < 3.0 X 109/L
    - Total bilirubin >= 20 micromol/liter (1.17mg/dl); except for subjects with isolated elevation of indirect bilirubin relating to Gilbert syndrome
    - Hemoglobin < 80 gram/liter
    - Platelets < 100,000/μL

Note: The laboratory markers of e.g. AST and ALT are deliberately specified relative to upper limit of normal as these values (and units used) differ between the different labs within the USA and across the study sites in the USA, Canada and Europe.

5.3 LIFESTYLE CONSIDERATIONS

Not applicable.

5.4 SCREEN FAILURES

Screen failures are defined as participants who consent to participate in the clinical trial but are not subsequently randomly assigned to the study intervention or entered in the study. A minimal set of screen failure information is required to ensure transparent reporting of screen failure participants, to meet the Consolidated Standards of Reporting Trials (CONSORT) publishing requirements and to respond to queries from regulatory authorities. Minimal information includes demography, screen failure details, eligibility criteria, and any serious adverse event (SAE).

Individuals who do not meet the criteria for participation in this trial (screen failure) because of incomplete clinical data or recent *Clostridium difficile* infection positivity may be rescreened. Rescreened participants should be assigned the same participant number as for the initial screening.

5.5 STRATEGIES FOR RECRUITMENT AND RETENTION

Potential participants will be identified by their treating gastroenterologist within 1 of 8 participating centres across the U.S.A., Canada, the Netherlands, and Israel. The study sites are pediatric gastroenterology clinics and participants will be recruited from patients who are undergoing diagnostic procedures and receiving medical treatment for Crohn's disease, as per standard of care.

For this pilot study, up to 5 participants from each site will be recruited until a total of 20 subjects. The treating gastroenterologist will ask the patient and their parent/guardian if they are interested in hearing more about the study and will obtain their verbal consent to be approached by a member of the study staff. The study staff member will explain the study and conduct and informed consent discussion with the potential participant and their parent/guardian. The treating gastroenterologist will be available to address any questions or concerns that the potential participant and their parent/guardian may have about participating in the study.

Study visits and procedures are timed to coincide with regularly scheduled follow-up visits and collection of biospecimens that would occur as per standard of care for children and adolescents being treated for active Crohn's disease. This design is expected to minimize the burden of study participation for the subject and their parent/guardian and to improve the adherence to the study visit schedule. If needed, a study timepoint window of +/- 5 days is allowed for all visits/questionnaires/sampling.

Direct incentives will not be offered to participants or their parent/guardian.

6 STUDY INTERVENTION

6.1 STUDY INTERVENTION(S) ADMINISTRATION

6.1.1 STUDY INTERVENTION DESCRIPTION

The antibiotics to be used in this study are azithromycin and metronidazole. Both drugs are FDA-approved and commercially available, most recent product labels are available at the following URLs:

Azithromycin:
https://www.accessdata.fda.gov/drugsatfda_docs/label/2017/050670s032,050710s046,050711s043,050784s030lbl.pdf

Metronidazole:
https://www.accessdata.fda.gov/drugsatfda_docs/label/2015/012623s066lbl.pdf

Azithromycin (also Zithromax, Zmax) is a macrolide antibacterial drug indicated for mild to moderate infections caused by designated, susceptible bacteria:

- Acute bacterial exacerbations of chronic bronchitis in adults
- Acute bacterial sinusitis in adults
- Uncomplicated skin and skin structure infections in adults
- Urethritis and cervicitis in adults
- Genital ulcer disease in men
- Acute otitis media in pediatric patients
- Community-acquired pneumonia in adults and pediatric patients
- Pharyngitis/tonsillitis in adults and pediatric patients

Metronidazole (also Flagyl) is an oral formulation of the synthetic nitroimidazole antimicrobial, 2-methyl-5-nitro-1H-imidazole-1-ethanol indicated for infections strongly suspected to be caused by bacteria, particularly in the treatment of serious infections caused by susceptible anaerobic bacteria.

- Symptomatic or asymptomatic Trichomoniasis (T. vaginalis) infection in females and male and asymptomatic sexual partners. T
- Acute intestinal amebiasis (amebic dysentery) and amebic liver abscess.
- Anaerobic bacterial infections, in a mixed aerobic and anaerobic infection, antimicrobials appropriate for the treatment of the aerobic infection should be used in addition to metronidazole.
    - INTRA-ABDOMINAL INFECTIONS, including peritonitis, intra-abdominal abscess, and liver abscess, caused by Bacteroides species including the B. fragilis group (B. fragilis, B. distasonis, B. ovatus, B. thetaiotaomicron, B. vulgatus), Clostridium species, Eubacterium species, Peptococcus species, and Peptostreptococcus species.
    - SKIN AND SKIN STRUCTURE INFECTIONS caused by Bacteroides species including the B. fragilis group, Clostridium species, Peptococcus species, Peptostreptococcus species, and Fusobacterium species.
    - GYNECOLOGIC INFECTIONS, including endometritis, endomyometritis, tubo-ovarian abscess, and postsurgical vaginal cuff infection, caused by Bacteroides species including

the B. fragilis group, Clostridium species, Peptococcus species, Peptostreptococcus species, and Fusobacterium species.
- BACTERIAL SEPTICEMIA caused by Bacteroides species including the B. fragilis group and Clostridium species.
- BONE AND JOINT INFECTIONS, (as adjunctive therapy), caused by Bacteroides species including the B. fragilis group.
- CENTRAL NERVOUS SYSTEM (CNS) INFECTIONS, including meningitis and brain abscess, caused by Bacteroides species including the B. fragilis group.
- LOWER RESPIRATORY TRACT INFECTIONS, including pneumonia, empyema, and lung abscess, caused by Bacteroides species including the B. fragilis group.
- ENDOCARDITIS caused by Bacteroides species including the B. fragilis group.

6.1.2 DOSING AND ADMINISTRATION

Dosage
Azithromycin will be administered at a dose of 7.5mg/kg to a maximum of 500mg/day for 5 consecutive days per week for the first 4 weeks and then 3 consecutive days/week for 4 weeks.

Metronidazole will be administered 10mg/kg twice daily (20mg/kg/day) to a maximum of 1000mg/day for 8 weeks.
Patients who suspect they are intolerant to the medications are advised to contact the study coordinator regarding any new symptoms (predefined as new onset of emesis, significant nausea, abdominal pain or diarrhoea).

At the discretion of the responsible physician, patients can be instructed to reduce the dose of metronidazole by 25% to 15mg/kg/day and divide the frequency to 3 times daily. Any dosage change will be appropriately documented in the CRF.

Formulations
Azithromycin and metronidazole are both available in 250mg tablets. Azithromycin is also commercially available as an oral suspension. Metronidazole is not commercially available as an oral suspension, but a suspension can be prepared by a pharmacy from 250mg tablets.

6.2 PREPARATION/HANDLING/STORAGE/ACCOUNTABILITY

6.2.1 ACQUISITION AND ACCOUNTABILITY

The investigational drugs will be acquired at each site independently, and labeled according to FDA labeling requirements found in 21CFR 312.6. The investigational Pharmacy at each site will dispense study drugs following enrollment and randomization according to study protocol. For Azithromycin 250 mg in tablets and suspension are available. Metronidazole is available in 250 mg tablets and pharmacies can also prepare an extemporaneous compound of the Metronidazole liquid (to be refrigerated).

Each of the participating study sites will dispense and label study drugs according to local guidelines. For example, at UNC, Vestigo software is used for the management of inventory and accountability as well as labeling the product with an outpatient label. Return of unused study drug and accountability on those returns will be performed using this same software system.

Subjects will be seen in dedicated outpatient clinics during this study and the local study coordinator will be delivering the appropriately dosed and labelled study drugs to the child/parent and explaining the medication and use and storage to them.

6.2.2 FORMULATION, APPEARANCE, PACKAGING, AND LABELING

Azithromycin and metronidazole are both available in 250mg tablets. Azithromycin is also commercially available as an oral suspension as 100 mg/5 mL. Metronidazole is not commercially available as an oral suspension, but a suspension can be prepared by a pharmacy from 250mg tablets.

Both drugs are FDA-approved and commercially available, most recent product labels are available at the following URLs:

https://www.accessdata.fda.gov/drugsatfda_docs/label/2017/050670s032,050710s046,050711s043,050784s030lbl.pdf

https://www.accessdata.fda.gov/drugsatfda_docs/label/2015/012623s066lbl.pdf

The supplier of the drug may differ from the label provided, but the route, administration, and product information will be the same.

6.2.3 PRODUCT STORAGE AND STABILITY

Azithromycin tablets should be stored between 15° to 30° C (59° to 86° F). Metronidazole tablets should be stored below 77°F (25°C) and protected from light.

Azithromycin for oral suspension is supplied to provide 100 mg/5 mL or 200 mg/5 mL suspension. Dry powder should be stored below 30°C (86°F). Constituted suspension should be stored between 5° to 30°C (41° to 86°F) and discarded when full dosing is completed.

Metronidazole is not commercially available as an oral suspension, but a suspension can be prepared by a pharmacy from 250mg tablets (to be refrigerated).

Both drugs are FDA-approved and commercially available, most recent product labels are available at the following URLs:

https://www.accessdata.fda.gov/drugsatfda_docs/label/2017/050670s032,050710s046,050711s043,050784s030lbl.pdf

https://www.accessdata.fda.gov/drugsatfda_docs/label/2015/012623s066lbl.pdf

The supplier of the drug may differ from the label provided, but the route, administration, and product information will be the same.

6.2.4 PREPARATION

Azithromycin and metronidazole are both available in 250mg tablets. Azithromycin is also commercially available as an oral suspension as 100 mg/5 mL. Metronidazole is not commercially available as an oral suspension, but a suspension can be prepared by a pharmacy from 250mg tablets. Pharmacies at each of the participating study sites will dispense and label study drugs according to local policies.

Both drugs are FDA-approved and commercially available, most recent product labels are available at the following URLs:

https://www.accessdata.fda.gov/drugsatfda_docs/label/2017/050670s032,050710s046,050711s043,050784s030lbl.pdf

https://www.accessdata.fda.gov/drugsatfda_docs/label/2015/012623s066lbl.pdf

The supplier of the drug may differ from the label provided, but the route, administration, and product information will be the same.

6.3 MEASURES TO MINIMIZE BIAS: RANDOMIZATION AND BLINDING

We will randomly allocate the subjects who are identified as having a relapse-associated microbiome into experimental treatment or control arms. The randomization process and creation of randomization envelopes are performed by the study coordinator at the Amsterdam UMC, location AMC. To ensure 1:1 randomization, blocks of 2 will be generated by using CastorEDC for patients randomized to group A. The same number of patients will be randomly allocated in the cohort groups A1 and A2.

Once the temporary ID has been matched with the participant's Study ID, a randomization pdf is created for the Study ID number, with the treatment group assignment and emailed to the local study-coordinator. The study coordinator will then prepare a sealed randomization envelope with a card stating whether they will take antibiotics or not to take antibiotics. The study coordinator will give this envelope to the participant & family to open this envelope in the presence of the local site-study coordinator. The physician will be informed of the outcome of the randomization. A prescription will then be issued by the treating physician. The local site-study coordinator will verify that each patient understands the instructions.

This study will not be blinded.

6.4 STUDY INTERVENTION COMPLIANCE

Subjects in the antibiotic treatment arm will be asked to complete a medication calendar to record the dose and any problems encountered while administering the dose (e.g. vomiting). Study staff will monitor adherence by counting tablets returned at the 12 week visit.

6.5 CONCOMITANT THERAPY

All subjects in this study are asked to continue their standard of care (SOC) treatment for CD as prescribed by their treating gastroenterologist.

For this protocol, a prescription medication is defined as a medication that can be prescribed only by a properly authorized/licensed clinician. Medications to be reported in the Case Report Form (CRF) are concomitant prescription medications (e.g. antacid medication, including additional clinically indicated antibiotics or anti-fungals), over-the-counter medications and supplements.

Patients taking over-the-counter prebiotics and/or probiotics will be counseled by their treating gastroenterologist at the time of enrolment and recommended to stop taking pre/probiotics by the week 0 study visit, and to refrain from using probiotics for the duration of the study (Week 52). If patients choose to continue using pre-and/or pro-biotics, this will be recorded on the CRF.

6.5.1 RESCUE MEDICINE

Not applicable.

7 STUDY INTERVENTION DISCONTINUATION AND PARTICIPANT DISCONTINUATION/WITHDRAWAL

7.1 DISCONTINUATION OF STUDY INTERVENTION

Discontinuation of the antibiotic treatment between weeks 4 and 12 does not mean discontinuation of the study. A participant who discontinues the antibiotic intervention will be moved into the Group A control group (Group A1) and receive SOC, but these patients will not be included in the analysis of Group A1 to avoid selection bias as a patient who discontinues treatment in week 11 may differ from a patient who discontinued in week 5. Any patients discontinuing treatment will therefore be reported separately.
The remaining study procedures should be completed as indicated by the study protocol. If a clinically significant finding is identified (including, but not limited to changes from baseline) after enrollment, the investigator or qualified designee will determine if any change in participant management is needed. Any new clinically relevant finding will be reported as an adverse event (AE). Specific criteria for discontinuation due to known or potential AE associated with the study intervention are provided in section 7.2.

7.2 PARTICIPANT DISCONTINUATION/WITHDRAWAL FROM THE STUDY

Participants are free to withdraw from participation in the study at any time for any reason if they wish to do so without any consequences. An investigator may discontinue or withdraw a participant from the study for the following reasons:

- Pregnancy
- Significant study intervention non-compliance
- Occurrence of a clinical AE known to be associated with study intervention including new onset peripheral neuropathy, new onset seizures, new onset clinically significant leukopenia, new-onset QT prolongation, evidence of hepatotoxicity, allergic reaction. Criteria for stopping investigational therapy due to potential hepatotoxicity include any of the following laboratory abnormalities:
    - ALT or AST >8xULN
    - ALT or AST >5xULN for more than 2 weeks
    - ALT or AST >3xULN and (TBL >2xULN or INR >1.5)
    - ALT or AST >3xULN with the appearance of fatigue, nausea, vomiting, right upper quadrant pain or tenderness, fever, rash, and/or eosinophilia (>5%)

  Should any of these laboratory abnormalities occur, the participant will discontinue investigational therapy and undergo appropriate evaluation for other causes of abnormal liver function tests. The participant must be followed until resolution or stabilization and the results of the evaluation for alternate causes of abnormal liver enzymes will be documented in the Reason for Withdrawal Case Report Form (CRF)
- If any other clinical adverse event, laboratory abnormality, or other medical condition or situation occurs such that continued participation in the study would not be in the best interest of the participant
- Disease progression which requires discontinuation of the study intervention
- If the participant meets an exclusion criterion (either newly developed or not previously recognized) that precludes further study participation

The reason for participant discontinuation or withdrawal from the study will be recorded on the Reason for Withdrawal CRF. Subjects who withdraw during the pilot study will try to be replaced.

7.3 LOST TO FOLLOW-UP

A participant will be considered lost to follow-up if he or she fails to return for 2 consecutive scheduled visits as well as the Week 52 follow-up visit, and is unable to be contacted by the study site staff.

The following actions must be taken if a participant fails to return to the clinic for a required study visit:

- The site will attempt to contact the participant and reschedule the missed visit and counsel the participant on the importance of maintaining the assigned visit schedule and ascertain if the participant wishes to and/or should continue in the study.
- Before a participant is deemed lost to follow-up, the investigator or designee will make every effort to regain contact with the participant (where possible, 3 telephone calls and, if necessary,

a certified letter to the participant's last known mailing address or local equivalent methods). These contact attempts should be documented in the participant's medical record or study file.

- Should the participant continue to be unreachable, he or she will be considered to have withdrawn from the study with a primary reason of lost to follow-up.

8 STUDY ASSESSMENTS AND PROCEDURES

8.1 EFFICACY ASSESSMENTS

History and Physical exam – These will be performed as per the standard of clinical care including height (height velocity), weight and perianal inspection. The primary outcome variable will be whether sustained remission at 52 weeks after starting standard of care induction therapy is achieved. Sustained remission is not defined by PCDAI alone; but rather by PCDAI ≤ 10 at week 52 AND not having need of re-induction for clinical flare (new course of EEN, need to start steroids), no steroid dependence, no biologic (anti-TNF) use, and no intestinal surgery by 12 months. As secondary outcome variables of efficacy, longitudinal (clustered and with respect to baseline) changes in disease activity indices components, and inflammatory markers in blood and stool, will be collected including the PCDAI score (0-100), normalization of CRP (<0.5mg/dL or the local reference range as applicable), fecal calprotectin (microgram/g) and Delta-fecal calprotectin before and after the study intervention. In addition, longitudinal (clustered and with respect to baseline) changes of Patient-reported outcome will be collected (IMPACT (self-report, ages 9-17 years).

Blood samples – Blood samples will be drawn as per the standard of clinical care including erythrocyte sedimentation rate (ESR), C-reactive protein, albumin, alanine transaminase (ALT), aspartate transaminase (AST), bilirubin, Urea (Blood Urea Nitrogen (BUN) and Creatinine,)and complete blood count (including hematocrit). These blood results, provided by the local clinical labs, will be required to calculate the disease activity index and monitor for toxicity due to methotrexate.

Stool samples – For assessment of inclusion criteria for Screening, stool samples will be obtained for local testing for the presence of toxigenic *C. difficile*. This sample may be a pre-consent sample collected as standard of care. Study stool samples will be collected at each study visit and split into two aliquots; one for Fecal calprotectin testing and one for microbiome analysis at the central laboratory Dalhousie University (Integrated Microbiome Resource) as coordinated from Amsterdam UMC.

Special assays - DNA sequencing and microbiome analysis: Identification of favorable vs unfavorable (Proteobacteria-rich) signature: the stool for analysis will be collected at baseline for screening (in children undergoing endoscopic investigations as decided by the responsible physician, stool will be obtained before the bowel prep for diagnostic endoscopy or starting from 48 hours after an endoscopy), in a dedicated kit (e.g. DNAGenotek) with solution buffer. Analysis of the V4-V5 16S region by a blinded bioinformatician (labelling the patients using Bayesian analysis (posterior probability >30% of carrying the Proteobacteria-rich, unfavorable signature) within 2-3 weeks, in order to allow participants to be randomized to either adjuvant antibiotics or no additional treatment, by the end of week 4 from baseline.

8.2 SAFETY AND OTHER ASSESSMENTS

Electrocardiograms (ECGs): ECG is for screening purposes only to identify prolongation of QT (QTc) corrected for age appropriate norms. If there is prolongation, as identified by each responsible physician, then the responsible physician is responsible for communicating this finding with the patient and family and arranging appropriate follow-up with cardiology. Patients found to have QT prolongation will not be treated with investigational therapy and appropriate guidance will be given regarding avoiding other medication with the potential of QTc prolongation (e.g. avoiding ondansetron). Further, all patients randomized to group A2 will receive a follow-up ECG 2-4 weeks after the initiation of the study drug to ensure that new-onset QT prolongation is not occurring as a result of azithromycin treatment. If clinically significant QT prolongation is detected at follow-up ECG, patients should discontinue investigational therapy. If the subject is not in clinical remission at week 4, then the responsible physician will decide whether azithromycin/metronidazole can be started as additional induction therapy.

Blood samples: Blood drawn as per the standard of clinical care will be tested for alanine transaminase (ALT), aspartate transaminase (AST), bilirubin, BUN and Creatinine by the local clinical labs to monitor for toxicity due to methotrexate.

Urine pregnancy tests: A urine pregnancy test will be administered at screening and repeated at week 4 prior to the initiation of methotrexate in post-menarchal female patients. These tests will be used to prevent exposure of participants to potential embryotoxic/teratogenic effects of methotrexate. Counselling and reinforcement of these potential risks and the importance of pregnancy prevention will be included at every study visit. Post-menarchal patients will be counselled about the use of birth control to prevent unplanned pregnancy while taking methotrexate during this study period.

8.3 ADVERSE EVENTS AND SERIOUS ADVERSE EVENTS

8.3.1 DEFINITION OF ADVERSE EVENTS (AE)

Adverse event means any untoward medical occurrence associated with the use of an intervention in humans, whether or not considered intervention-related (21 CFR 312.32 (a)).

8.3.2 DEFINITION OF SERIOUS ADVERSE EVENTS (SAE)

An adverse event (AE) or suspected adverse reaction is considered "serious" if, in the view of either the investigator or sponsor, it results in any of the following outcomes: death, a life-threatening adverse event, inpatient hospitalization or prolongation of existing hospitalization, a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions, or a congenital anomaly/birth defect.

Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the

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

8.3.3 CLASSIFICATION OF AN ADVERSE EVENT

8.3.3.1 SEVERITY OF EVENT

For adverse events (AEs) not included in the protocol defined grading system, the following guidelines will be used to describe severity.

- **Mild** – Events require minimal or no treatment and do not interfere with the participant's daily activities.
- **Moderate** – Events result in a low level of inconvenience or concern with the therapeutic measures. Moderate events may cause some interference with functioning.
- **Severe** – Events interrupt a participant's usual daily activity and may require systemic drug therapy or other treatment. Severe events are usually potentially life-threatening or incapacitating. Of note, the term "severe" does not necessarily equate to "serious".

8.3.3.2 RELATIONSHIP TO STUDY INTERVENTION

All adverse events (AEs) must have their relationship to study intervention assessed by the clinician who examines and evaluates the participant based on temporal relationship and his/her clinical judgment. The degree of certainty about causality will be graded using the categories below. In a clinical trial, the study product must always be suspect.

- **Related** – The AE is known to occur with the study intervention, there is a reasonable possibility that the study intervention caused the AE, or there is a temporal relationship between the study intervention and event. Reasonable possibility means that there is evidence to suggest a causal relationship between the study intervention and the AE.
- **Not Related** – There is not a reasonable possibility that the administration of the study intervention caused the event, there is no temporal relationship between the study intervention and event onset, or an alternate etiology has been established.

8.3.3.3 EXPECTEDNESS

The investigator will be responsible for determining whether an adverse event (AE) is expected or unexpected. An AE will be considered unexpected if the nature, severity, or frequency of the event is not consistent with the risk information previously described for the study intervention.

8.3.4 TIME PERIOD AND FREQUENCY FOR EVENT ASSESSMENT AND FOLLOW-UP

The occurrence of an adverse event (AE) or serious adverse event (SAE) may come to the attention of study personnel during study visits of a study participant presenting for medical care, or upon review by a study monitor.

All AEs including local and systemic reactions not meeting the criteria for SAEs will be captured on the appropriate case report form (CRF). Information to be collected includes event description, time of onset, clinician's assessment of severity, relationship to study product (assessed only by those with the training and authority to make a diagnosis), and time of resolution/stabilization of the event. All AEs occurring while on study must be documented appropriately regardless of relationship. All AEs will be followed to adequate resolution.

Any medical condition that is present at the time that the participant is screened will be considered as baseline and not reported as an AE. However, if the study participant's condition deteriorates at any time during the study, it will be recorded as an AE.

Changes in the severity of an AE will be documented to allow an assessment of the duration of the event at each level of severity to be performed. AEs characterized as intermittent require documentation of onset and duration of each episode.

The study clinician will record all reportable events with start dates occurring any time after informed consent is obtained until 7 (for non-serious AEs) or 30 days (for SAEs) after the last day of study participation. At each study visit, the investigator will inquire about the occurrence of AE/SAEs since the last visit. Events will be followed for outcome information until resolution or stabilization.]

8.3.5 ADVERSE EVENT REPORTING

Details of all adverse events reported spontaneously by subjects or observed by the investigator or study clinician will be recorded in appropriate adverse event report forms and reported to the principal investigator and the responsible medical doctor. All adverse events observed will be recorded separately.

8.3.6 SERIOUS ADVERSE EVENT REPORTING

The study clinician will immediately report to the sponsor any serious adverse event, whether or not considered study intervention related, including those listed in the protocol or investigator brochure and must include an assessment of whether there is a reasonable possibility that the study intervention caused the event. Study outcome variables that are serious adverse events (e.g., all-cause mortality) must be reported in accordance with the protocol unless there is evidence suggesting a causal relationship between the study intervention and the event (e.g., death from anaphylaxis). In that case, the investigator must immediately report the event to the sponsor.

All serious adverse events (SAEs) will be followed until satisfactory resolution or until the site investigator deems the event to be chronic or the participant is stable. Other supporting documentation of the event may be requested by the Data Coordinating Center (DCC)/study sponsor and should be provided as soon as possible.

As per C.05.014 (1) of the FDR, the study sponsor will be responsible for notifying FDA/Health Canada/CCMO (Netherlands) of any unexpected fatal or life-threatening suspected adverse reaction as soon as possible, but in no case later than 7 calendar days after the sponsor's initial receipt of the

information. Within 8 days after having initially informed FDA/Health Canada/CCMO of the fatal or life-threatening SAE, the sponsor will submit as complete a report as possible. Follow-up reports of fatal or life-threatening reactions must include an assessment of the importance and implication of the findings, including relevant previous experience with the same or similar drugs.

Where the SAE is neither fatal nor life-threatening, the sponsor will inform FDA/Health Canada/CCMO (Netherlands) within 15 days after becoming aware of the information.

SUSAR´s that have occurred in all participating countries should be reported by the sponsor. Further steps will be taken according to local legislation.

8.3.7 REPORTING EVENTS TO PARTICIPANTS

Participants will be notified directly be a study staff member.

8.3.8 EVENTS OF SPECIAL INTEREST

Not applicable

8.3.9 REPORTING OF PREGNANCY

If a participant becomes pregnant while taking the antibiotic intervention, they will discontinue and will be withdrawn from the study.

Animal reproduction studies have been performed in rats and mice at doses up to moderately maternally toxic dose concentrations (i.e., 200 mg/kg/day) (2-4 times the human daily dose) and no evidence of harm to the fetus due to azithromycin was found.[39] Reproduction studies for metronidazole have been performed in rates, rabbits, and mice at doses similar to the maximum recommended human dose and have found no evidence of harm to the fetus due to metronidazole.[40] However, there are no adequate or well-controlled studies of azithromycin or metronidazole use in pregnant women.

8.4 UNANTICIPATED PROBLEMS

8.4.1 DEFINITION OF UNANTICIPATED PROBLEMS (UP)

The Office for Human Research Protections (OHRP) considers unanticipated problems involving risks to participants or others to include, in general, any incident, experience, or outcome that meets **all** of the following criteria:

- Unexpected in terms of nature, severity, or frequency given (a) the research procedures that are described in the protocol-related documents, such as the Institutional Review Board (IRB)-approved research protocol and informed consent document; and (b) the characteristics of the participant population being studied;

- Related or possibly related to participation in the research ("possibly related" means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research); and
- Suggests that the research places participants or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

8.4.2 UNANTICIPATED PROBLEM REPORTING

The investigator will report unanticipated problems (UPs) to the reviewing Institutional Review Board (IRB) and to the Data Coordinating Center (DCC)/lead principal investigator (PI). The UP report will include the following information:

- Protocol identifying information: protocol title and number, PI's name, and the IRB project number;
- A detailed description of the event, incident, experience, or outcome;
- An explanation of the basis for determining that the event, incident, experience, or outcome represents an UP;
- A description of any changes to the protocol or other corrective actions that have been taken or are proposed in response to the UP.

To satisfy the requirement for prompt reporting, UPs will be reported using the following timeline:

- UPs that are serious adverse events (SAEs) will be reported to the IRB and to the DCC/study sponsor within 7 calendar days of the investigator becoming aware of the event.
- Any other UP will be reported to the IRB and to the DCC/study sponsor within <insert timeline in accordance with policy> of the investigator becoming aware of the problem.
- All UPs should be reported to appropriate institutional officials (as required by an institution's written reporting procedures), the supporting agency head (or designee), and the Office for Human Research Protections (OHRP) within <insert timeline in accordance with policy> of the IRB's receipt of the report of the problem from the investigator.

8.4.3 REPORTING UNANTICIPATED PROBLEMS TO PARTICIPANTS

Participants will be notified directly by a study staff member.

9 STATISTICAL CONSIDERATIONS

9.1 STUDY OUTCOME VARIABLES

The primary outcome evaluation:

The primary efficacy outcome variable is being in sustained remission defined as PCDAI ≤ 10 at 52 weeks from start of therapy AND without steroid dependence and without the need for repeat induction therapy, anti-TNFα, or surgery by 52 weeks from start of therapy.

The secondary outcome evaluations at each study visit include:

- Mean change in PCDAI score from baseline to weeks 4, 12, 24, 36, and 52.
- Mean change in Inflammatory markers: CRP and fecal calprotectin from baseline to weeks 4, 12, 24, 36, and 52.
- Mean change in PRO score (IMPACT questionnaire) from baseline to weeks 4, 12, 24, 36, and 52.
- Improvement (yes vs no) of disease activity (change in PCDAI < 0) rate at weeks 4, 12, 24, 36, and 52.
- Improvement (yes vs no) of C-Reactive Protein (change in CRP < 0 mg/dL) and fecal calprotectin (change in FCP < 0 µg/g) rate at weeks 4, 12, 24, 36, and 52.
- Normalization (yes vs no) of disease activity (PCDAI < 10) rate at weeks 4, 12, 24, 36, and 52.
- Normalization (yes vs no) of C-Reactive Protein (CRP; < 0.5 mg/dL) and fecal calprotectin (FCP; < 250 µg/g) rate at weeks 4, 12, 24, 36, and 52.

9.2 SAMPLE SIZE DETERMINATION

Based on published literature, 50% of children will require repeat induction/treatment escalation by the end of the first year after diagnosis.[43-45] 25 children carrying the high-risk microbiome signature will need to be randomized to each study arm (A1 or A2) (10% drop out assumed) in the larger trial following on from this pilot trial, assuming 80% power, to reduce the risk of flaring from 80% to 40%. These calculations are based on assumptions of relapse risk as no prospective microbiome data are available to inform this risk prediction of an unfavourable disease course.
In this pilot trial, 20 children will be enrolled to assess feasibility. The clinical data about relapse risk were not used to determine this sample size for the pilot trial. The 20 children of the pilot study will be allocated to either group A or group B, based on baseline microbiome data, unless the subject does not achieve clinical remission at week 4. If the subject does not achieve clinical remission (PCDAI less than or equal to 10) then the responsible physician can decide to start azithromycin/metronidazole for 8 weeks as additional induction therapy in Group C. Randomization will determine whether patients in group A, will be given SOC (A1) or SOC+antibiotics (A2).

9.3 POPULATIONS FOR ANALYSES

- Intention-to-Treat (ITT) Analysis Dataset will include all randomized participants: we will compare group A1 vs. group A2 for the primary analyses. In secondary/exploratory outcome analyses, we will compare the effects of treatment with antibiotics on the microbiome to assess whether indeed the signature of group A (A2) can be modified to move closer to group B.
  If this is the case, then (A2+B) vs. A1, will be compared. If the microbiome shifts associated with antibiotic use do not make A2 more similar to group B, then we will compare A1+B vs. A2 to assess whether there is an additional immune-regulatory effect of azithromycin (in combination with metronidazole) to influence disease flare risk. Group C samples will be analysed as a separate group in an observational study arm.

- Modified Intention-to-Treat Analysis Dataset will include participants who took the antibiotic intervention for at least 48 hours and had at least 6 months of follow-up outcome data.

- Safety Analysis Dataset will include participants who took at least 1 week of study intervention for whom safety analyses will be conducted

- Per-Protocol Analysis Dataset will include participants in the full analysis (ITT) set who took at least 2 weeks of study intervention during the treatment period and complied with the protocol sufficiently to ensure that these data would be likely to represent the effects of study intervention according to the underlying scientific model

9.4 STATISTICAL ANALYSES

9.4.1 GENERAL APPROACH

All summary tables will be presented by treatment group (Group A1, A2, and B) and, where applicable, by study visits. For descriptive statistics, continuous variables will be summarized with the mean, median, standard deviation, minimum and maximum. Categorical variables will be presented as counts and proportions expressed as a percentage. In addition, summary figures will be generated for longitudinal data to display change in mean or proportion over time.

Missing values in continuous variables will not be imputed. Missing values in categorical variables will be imputed as non-responders.

All tests will be two-sided and considered significant at $P<0.05$. Hypothesis tests that are not statistically significant will be deemed inconclusive. No adjustments to the test-wise error rate will be made.

9.4.2 ANALYSIS OF THE PRIMARY OUTCOME VARIABLES

The primary outcome variable is sustained remission defined as PCDAI less than or equal to 10 at 52 weeks AND no need of re-induction for clinical flare (new course of nutritional therapy, need to restart steroids), steroid dependence, biologic (anti-TNF/ustekinumab/vedolizumab) use, and/or intestinal surgery by 12 months. The outcome variable is categorical and measured at a single outcome variable (52 weeks). The number and percentage of patients with a relapse-associated gut microbiome profile will be summarized by group and the difference in sustained remission rate will be compared using a Fisher's exact test (comparing group A1 with A2).

The analysis of the primary efficacy parameter will be based on the ITT population. A sensitivity analysis using the mITT population will also be conducted for confirmatory purposes.

9.4.3 ANALYSIS OF THE SECONDARY OUTCOME VARIABLE(S)

In secondary outcome analyses, the effects of treatment with antibiotics on the microbiome will be assessed to determine whether the signature of group A2 can be modified to move closer to group B.

If this is the case, then groups A2 and B will be pooled and compared against A1. If the microbiome shifts associated with antibiotic use do not make A2 more similar to group B, then groups A1 and B will be pooled and compared against A2 to assess whether there is an additional immune-regulatory effect of azithromycin (in combination with metronidazole) to influence disease flare risk.

Data for secondary outcome variable analysis are independent of the primary outcome variable and will be collected at each study visit.

Continuous secondary outcomes will be summarized at each visit for both the observed values and the changes from baseline. A linear mixed model with timepoint, group, their interaction and baseline as covariates will be used to assess the difference in means between groups at each timepoint. An AR(1) covariance structure will be assumed and the Kenward-Roger degrees of freedom approximation will be utilized. Least squares estimates for the population mean and standard deviation will be provided at each assessment timepoint by group. Missing values will not be imputed. Due to the exploratory nature of the assessments, no correction for multiplicity will be made.

Categorical secondary outcomes variables will be analyzed similarly to the primary outcome (Section 9.4.2).

The analysis of the secondary efficacy parameters will be based on the ITT population.

9.4.4 SAFETY ANALYSES

This study is not evaluating a formal safety outcome variable.

9.4.5 DEMOGRAPHIC AND BASELINE DESCRIPTIVE STATISTICS

Demographic and baseline characteristics will be based on the Intent-to-Treat (ITT) population. For evaluations which are performed at both the screening and baseline visits (Visit 2), baseline values are to be taken from the baseline visit.

Baseline characteristics to be compared between treatments will include age, gender, age at diagnosis, disease location and behavior (classified using the Paris classification scheme), disease activity (PCDAI score), CRP values, FCP values, and anthropometrics including height and weight z-score.

9.4.6 PLANNED INTERIM ANALYSES

Not applicable

9.4.7 SUB-GROUP ANALYSES

A priori subgroup analyses are unlikely to be powered sufficiently in this pilot trial. We will assess the effect of different gender and clinical phenotype (e.g. isolated colonic Crohn's vs isolated ileal Crohn's vs

ileo-colonic involvement of the lower GI tract) although these are often influenced by disease severity at diagnosis and their effects may be confounded with the effects of the disease severity. All results will be considered exploratory.

9.4.8 TABULATION OF INDIVIDUAL PARTICIPANT DATA

Participant data will be recorded individually by measure and for each time point.

9.4.9 EXPLORATORY ANALYSES

Exploratory analyses will involve the microbiome sequence data obtained from stool samples collected during the study. Changes in microbiome taxonomic composition and genome (metagenome) composition will be compared to changes in disease activity measured at each study visit, while taking into account other clinical phenotypic data.

10 SUPPORTING DOCUMENTATION AND OPERATIONAL CONSIDERATIONS

10.1 REGULATORY, ETHICAL, AND STUDY OVERSIGHT CONSIDERATIONS

10.1.1 INFORMED CONSENT PROCESS

10.1.1.1 CONSENT/ASSENT AND OTHER INFORMATIONAL DOCUMENTS PROVIDED TO PARTICIPANTS

Consent forms describing in detail the study intervention, study procedures, and risks are given to the participant and written documentation of informed consent is required prior to starting intervention/administering study intervention. The following consent materials are submitted with this protocol:

Information and Authorization Form
Information and Consent Form
Assent Form

10.1.1.2 CONSENT PROCEDURES AND DOCUMENTATION

Informed consent is a process that is initiated prior to the individual's agreeing to participate in the study and continues throughout the individual's study participation. Consent forms will be Institutional Review Board (IRB)-approved and the participant will be asked to read and review the document. The investigator will explain the research study to the participant and answer any questions that may arise. A verbal explanation will be provided in terms suited to the participant's comprehension of the purposes, procedures, and potential risks of the study and of their rights as research participants. Participants will have the opportunity to carefully review the written consent form and ask questions prior to signing. The participants should have the opportunity to discuss the study with their family or surrogates or think about it prior to agreeing to participate. The participant will sign the informed consent document prior to any procedures being done specifically for the study. Participants must be informed that participation is voluntary and that they may withdraw from the study at any time, without

prejudice. A copy of the informed consent document will be given to the participants for their records. The informed consent process will be conducted and documented in the source document (including the date), and the form signed, before the participant undergoes any study-specific procedures. The rights and welfare of the participants will be protected by emphasizing to them that the quality of their medical care will not be adversely affected if they decline to participate in this study.

10.1.2 STUDY DISCONTINUATION AND CLOSURE

This study may be temporarily suspended or prematurely terminated if there is sufficient reasonable cause. Written notification, documenting the reason for study suspension or termination, will be provided by the suspending or terminating party to study participants, investigator, funding agency, the Investigational New Drug (IND) sponsor and regulatory authorities. If the study is prematurely terminated or suspended, the Principal Investigator (PI) will promptly inform study participants, the Institutional Review Board (IRB), and sponsor and will provide the reason(s) for the termination or suspension. Study participants will be contacted, as applicable, and be informed of changes to study visit schedule.

Circumstances that may warrant termination or suspension include, but are not limited to:

- Determination of unexpected, significant, or unacceptable risk to participants
- Demonstration of efficacy that would warrant stopping
- Insufficient compliance to protocol requirements
- Data that are not sufficiently complete and/or evaluable
- Determination that the primary outcome variable has been met
- Determination of futility

The study may resume once concerns about safety, protocol compliance, and data quality are addressed, and satisfy the sponsor, IRB and/or FDA/Health Canada.

10.1.3 CONFIDENTIALITY AND PRIVACY

Participant confidentiality and privacy is strictly held in trust by the participating investigators, their staff, and the sponsor(s) and their interventions. This confidentiality is extended to cover testing of biological samples and genetic tests in addition to the clinical information relating to participants. Therefore, the study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorized third party without prior written approval of the sponsor.

All research activities will be conducted in as private a setting as possible.

The study monitor, other authorized representatives of the sponsor, representatives of the Institutional Review Board (IRB), regulatory agencies or pharmaceutical company supplying study product may inspect all documents and records required to be maintained by the investigator, including but not limited to, medical records (office, clinic, or hospital) and pharmacy records for the participants in this study. The clinical study site will permit access to such records.

The study participant's contact information will be securely stored at each clinical site for internal use during the study. At the end of the study, all records will continue to be kept in a secure location for as long a period as dictated by the reviewing IRB, Institutional policies, or sponsor requirements.

Study participant research data, which is for purposes of statistical analysis and scientific reporting, will be transmitted to and stored at the Amsterdam UMC, location AMC (Amsterdam, The Netherlands). This will not include the participant's contact or identifying information. Rather, individual participants and their research data will be identified by a unique study identification number. The study data entry and study management systems used by clinical sites and by the Amsterdam UMC research staff will be secured and password protected. At the end of the study, all study databases will be de-identified and archived at the Amsterdam UMC, location AMC .

To further protect the privacy of study participants, a Certificate of Confidentiality will be issued by the National Institutes of Health (NIH). This certificate protects identifiable research information from forced disclosure. It allows the investigator and others who have access to research records to refuse to disclose identifying information on research participation in any civil, criminal, administrative, legislative, or other proceeding, whether at the federal, state, or local level. By protecting researchers and institutions from being compelled to disclose information that would identify research participants, Certificates of Confidentiality help achieve the research objectives and promote participation in studies by helping assure confidentiality and privacy to participants.

10.1.4 FUTURE USE OF STORED SPECIMENS AND DATA

Data collected for this study will be analyzed and stored at the Amsterdam UMC, location AMC . After the study is completed, the de-identified, archived data will be transmitted to and stored at the Amsterdam UMC, location AMC for use by other researchers including those outside of the study. Permission to transmit data to the Amsterdam UMC will be included in the informed consent.

With the participant's approval and as approved by local Institutional Review Boards (IRBs), de-identified biological samples will be stored at the Amsterdam UMC with the same goal as the sharing of data with the Amsterdam UMC. These samples could be used to research the causes of inflammatory bowel diseases (IBD), its complications and other conditions for which individuals with IBD are at increased risk, and to improve treatment. The Amsterdam UMC will also be provided with a code-link that will allow linking the biological specimens with the phenotypic data from each participant, maintaining the blinding of the identity of the participant.

During the conduct of the study, an individual participant can choose to withdraw consent to have biological specimens stored for future research. However, withdrawal of consent with regard to biospecimen storage may not be possible after the study is completed.

When the study is completed, access to study data and/or samples will be provided through the Amsterdam UMC, location AMC.

10.1.5 KEY ROLES AND STUDY GOVERNANCE

Principal Investigator	**Medical Monitor**
Johan Van Limbergen MD FRCPCH PhD	Anthony Otley MD MSc FRCPC
Amsterdam University Medical Centers – location AMC	IWK Health Centre, Halifax

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The study oversight committee has been convened by Crohn's Colitis Foundation and consists of internationally renowned pediatric IBD experts. For conduct of the study in Canada, a separate Health Canada application will be submitted. For participating centres in the Netherlands, local ethics committee applications will be filed. In Israel, conduct of the study has been submitted to the ministry of Health and will follow guidelines of Good Clinical Practice (GCP).

10.1.6 SAFETY OVERSIGHT

Safety oversight will be under the direction of a Data and Safety Monitoring Board (DSMB) composed of individuals with the appropriate expertise, including experts in clinical care and trials in pediatric gastroenterology and inflammatory bowel disease. Members of the DSMB should be independent from the study conduct and free of conflict of interest, or measures should be in place to minimize perceived conflict of interest. The DSMB will meet at least semiannually to assess safety and efficacy data on each arm of the study. The DMSB will operate under the rules of an approved charter that will be written and reviewed at the organizational meeting of the DSMB. At this time, each data element that the DSMB needs to assess will be clearly defined. The DSMB will provide its input to the study sponsor Dr. Johan Van Limbergen as well to Dr. Francisco Sylvester.

10.1.7 CLINICAL MONITORING

Clinical site monitoring is conducted to ensure that the rights and well-being of trial participants are protected, that the reported trial data are accurate, complete, and verifiable, and that the conduct of the trial is in compliance with the currently approved protocol/amendment(s), with International Conference on Harmonisation Good Clinical Practice (ICH GCP), and with applicable regulatory requirement(s).

- Centralized monitoring will be arranged by the PI in Amsterdam by means of review by an accredited study monitor, who is not a member of the research team, of the electronically recorded CRF (using RedCap) data from this pilot trial, after each site has enrolled (to ensure an initial assessment of each site's data quality) and at the end of the study. Local monitoring will be ensured, as indicated by the central study monitor on an as-needed basis, or by targeted review of copies of study documents shared electronically with the central monitor.
- Independent audits will not be conducted for this pilot trial.

10.1.8 QUALITY ASSURANCE AND QUALITY CONTROL

Each clinical site will perform internal quality management of study conduct, data and biological specimen collection, documentation and completion. An individualized quality management plan will be developed to describe a site's quality management.

Quality control (QC) procedures will be implemented beginning with the data entry system and data QC checks that will be run on the database will be generated. Any missing data or data anomalies will be communicated to the site(s) for clarification/resolution and will be documented in the study database.

Following written Standard Operating Procedures (SOPs), the monitors will verify that the clinical trial is conducted and data are generated and biological specimens are collected, documented (recorded), and reported in compliance with the protocol, International Conference on Harmonisation Good Clinical Practice (ICH GCP), and applicable regulatory requirements (e.g., Good Laboratory Practices (GLP), Good Manufacturing Practices (GMP)).

The investigational site will provide direct access to all trial related sites, source data/documents, and reports for the purpose of monitoring and auditing by the sponsor, and inspection by local and regulatory authorities.

The reasons for missing data values will be investigated and documented in the study database.

10.1.9 DATA HANDLING AND RECORD KEEPING

10.1.9.1 DATA COLLECTION AND MANAGEMENT RESPONSIBILITIES

Data collection is the responsibility of the clinical trial staff at the site under the supervision of the site investigator. The investigator is responsible for ensuring the accuracy, completeness, legibility, and timeliness of the data reported.

All source documents should be completed in a neat, legible manner to ensure accurate interpretation of data.

Hardcopies of the study visit worksheets will be provided for use as source document worksheets for recording data for each participant enrolled in the study. Data recorded in the electronic case report form (eCRF) derived from source documents should be consistent with the data recorded on the source documents.

Clinical data (including adverse events (AEs), concomitant medications, and expected adverse reactions data) and clinical laboratory data will be entered into a study-specific Castor (Research Electronic Data Capture) database (*www.castoredc.com*). The data system includes password protection and internal quality checks, such as automatic range checks, to identify data that appear inconsistent, incomplete, or inaccurate. Clinical data will be entered directly from the source documents.

10.1.9.2 STUDY RECORDS RETENTION

The sponsor will retain and maintain all record pertaining to the clinical trial for a period of 25 years [as per C.05.012 (4) of the FDR]

10.1.10 PROTOCOL DEVIATIONS

A protocol deviation is any noncompliance with the clinical trial protocol, International Conference on Harmonisation Good Clinical Practice (ICH GCP), or Manual of Procedures (MOP) requirements. The noncompliance may be either on the part of the participant, the investigator, or the study site staff. As a result of deviations, corrective actions are to be developed by the site and implemented promptly.

These practices are consistent with ICH GCP:

- 4.5 Compliance with Protocol, sections 4.5.1, 4.5.2, and 4.5.3
- 5.1 Quality Assurance and Quality Control, section 5.1.1
- 5.20 Noncompliance, sections 5.20.1, and 5.20.2.

It is the responsibility of the site investigator to use continuous vigilance to identify and report deviations within 30 working days of identification of the protocol deviation, or within 5 working days of the scheduled protocol-required activity. All deviations must be addressed in study source documents, reported to Crohn's & Colitis Foundation Program Official and Dr. Johan Van Limbergen. Protocol deviations must be sent to the reviewing Institutional Review Board (IRB) per their policies. The site investigator is responsible for knowing and adhering to the reviewing IRB requirements. Further details about the handling of protocol deviations will be included in the MOP.

10.1.11 PUBLICATION AND DATA SHARING POLICY

This study will be conducted in accordance with the following publication and data sharing policies and regulations:

National Institutes of Health (NIH) Public Access Policy, which ensures that the public has access to the published results of NIH funded research. It requires scientists to submit final peer-reviewed journal manuscripts that arise from NIH funds to the digital archive PubMed Central upon acceptance for publication.

This study will comply with the NIH Data Sharing Policy and Policy on the Dissemination of NIH-Funded Clinical Trial Information and the Clinical Trials Registration and Results Information Submission rule. As such, this trial will be registered at ClinicalTrials.gov, and results information from this trial will be submitted to ClinicalTrials.gov. In addition, every attempt will be made to publish results in peer-reviewed journals. Data from this study may be requested by other researchers for 5 years after the completion of the primary outcome variable by contacting Dr. Johan Van Limbergen (Amsterdam UMC).

In addition, this study will comply with the NIH Genomic Data Sharing Policy, which applies to all NIH-funded research that generates large-scale human or non-human genomic data, as well as the use of these data for subsequent research. Large-scale data include genome-wide association studies (GWAS), single nucleotide polymorphisms (SNP) arrays, and genome sequence, transcriptomic, epigenomic, and gene expression data.

10.1.12 CONFLICT OF INTEREST POLICY

The independence of this study from any actual or perceived influence, such as by the pharmaceutical industry, is critical. Therefore, any actual conflict of interest of persons who have a role in the design, conduct, analysis, publication, or any aspect of this trial will be disclosed and managed. Furthermore, persons who have a perceived conflict of interest will be required to have such conflicts managed in a way that is appropriate to their participation in the design and conduct of this trial. The study leadership in conjunction with the Crohn's & Colitis Foundation has established policies and procedures for all study group members to disclose all conflicts of interest and will establish a mechanism for the management of all reported dualities of interest.

10.2 ADDITIONAL CONSIDERATIONS

10.3 ABBREVIATIONS

AE	Adverse Event
ALT	Alanine Transaminase
ANCOVA	Analysis of Covariance
AST	Aspartate Transaminase
CD	Crohn's Disease
CFR	Code of Federal Regulations
CLIA	Clinical Laboratory Improvement Amendments
CMP	Clinical Monitoring Plan
COC	Certificate of Confidentiality
CONSORT	Consolidated Standards of Reporting Trials
CRF	Case Report Form
CRP	C-reactive Protein
DCC	Data Coordinating Center
DHHS	Department of Health and Human Services
DSMB	Data Safety Monitoring Board
DRE	Disease-Related Event
EC	Ethics Committee
ECG	Electrocardiogram
eCRF	Electronic Case Report Forms
EEN	Exclusive Enteral Nutrition
ESR	Erythrocyte sedimentation rate
FCP	Fecal calprotectin
FDR	Food and Drug Regulations
FFR	Federal Financial Report
GCP	Good Clinical Practice
GLP	Good Laboratory Practices
GMP	Good Manufacturing Practices
GWAS	Genome-Wide Association Studies
HIPAA	Health Insurance Portability and Accountability Act
HRQOL	Health-Related Quality of Life
IB	Investigator's Brochure
IBD	Inflammatory bowel disease
ICH	International Conference on Harmonisation
ICMJE	International Committee of Medical Journal Editors
IDE	Investigational Device Exemption
IMPACT	IBD Quality-of-Life Questionnaire
IND	Investigational New Drug Application
IRB	Institutional Review Board
ISM	Independent Safety Monitor
ISO	International Organization for Standardization
ITT	Intention-To-Treat
LSMEANS	Least-squares Means
MedDRA	Medical Dictionary for Regulatory Activities
MOP	Manual of Procedures
MSDS	Material Safety Data Sheet
MTX	Methotrexate
NCT	National Clinical Trial
NIH	National Institutes of Health

NIH IC	NIH Institute or Center
OHRP	Office for Human Research Protections
PCDAI	Pediatric Crohn's Disease Activity Index
PI	Principal Investigator
PRO	Patient-Reported Outcome
QA	Quality Assurance
QC	Quality Control
REDCap	Research Electronic Data Capture
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SMC	Safety Monitoring Committee
SOA	Schedule of Activities
SOC	Standard of Care
SOP	Standard Operating Procedure
TNF	Tumor Necrosis Factor
UP	Unanticipated Problem
US	United States

10.4 PROTOCOL AMENDMENT HISTORY

Version	Date	Description of Change	Brief Rationale
	August 2019	Only CDED+PEN as induction therapy Open label antibiotics (azithromycin/metronidazole) if not in clinical remission by week 4	Publication of CDED RCT and reduction of patients in pilot study to 20
Addendum	October 2020	Feasibility study clarification	Required by National Ethics Review Board in the Netherlands October 2020
1.8	March 2021	Adding study timepoint window of +/- 5 days in case needed	To facilitate planning of study visits
1.8	March 2021	Changed IWK health centre to Amsterdam UMC, location AMC as principal receiver of trial data and biological samples	PI moved to Amsterdam and trial will be coordinated from Amsterdam UMC, location AMC
1.8	March 2021	Updated participating sites	North Carolina Children's Hospital (US), Sheba Medical Centre (Israel), Location VUMC of Amsterdam UMC (The Netherlands) will no longer be participating.
1.8	March 2021	Changed medical monitor to Prof. Anthony Otley	Prof. Antony Otley (local PI IWK Canada) will be the new medical monitor as former medical monitor prof. Francisco Sylvester (UNC) will no longer be involved in the study.
1.8	May 2021	Updated trial monitor name to Alimentiv B.V.	Alimentiv B.V. is the new name of the PAZAZ trial monitor, formerly known as Robarts Clinical Trial Inc.
Addendum	May 2021	Specifying chosen medicinal products for the AmsterdamUMC.	Metronidazole 500 mg, Metronidazole 250 mg, Azitromycin 250 mg will be the medicinal products used in the AmsterdamUMC.
1.9	October 2021	Use CastorEDC randomization function instead of an additional separate program (SPSS)	To use only one program (CastorEDC) as eCRF and randomization tool to minimize mistakes

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12 ADDENDUM FOR THE NETHERLANDS (OCTOBER 2020, UPD. MAY 2021)

This addendum contains specific information regarding the pilot trial of the PAZAZ study and additional information for The Netherlands.

PAZAZ PILOT STUDY

Aim

The aim of this pilot study is *feasibility* for the protocol of a multinational microbiome-randomisation trial in which early microbiome diagnostic procedure helps with personalizing treatment in children with mild to moderate CD.

Rationale for Pilot study

We want to investigate the workability of a protocol with specific timeframes for analysis that have to be met in order to conduct the trial as described. This is especially important for logistics concerning baseline stool sample analysis, in which baseline stool samples have to be sent from every site to Halifax (Canada) to be analyzed and interpreted by a central analyst. Results have to be available at week 4 at the local study centers in order to perform correct treatment allocation.

Objectives and outcome variables

Adapted from section 3

Objectives	Outcome variables	Justification of outcome variables and remarks
Primary		
To evaluate the feasibility of a multicenter trial on different continents with treatment allocation at week 4 depending on stool sample results at baseline	Proportion of subjects that are successfully randomized in randomization procedure, proportion of patients per treatment arm, proportion of subjects that complete 1 year endpoint	For a potential RCT on full-scale we first need to investigate whether the protocol is feasible in terms of logistics and randomisation plan.
To evaluate the potential efficacy of personalized adjunctive antibiotic therapy in maintaining clinical remission in pediatric subjects undergoing SOC induction therapy for mild to moderate Crohn's disease who have a relapse-associated microbiome profile	Proportion of subjects in sustained remission at 52 weeks after starting standard of care induction therapy Sustained remission is defined PCDAI less than or equal to 10 AND no need of re-induction for clinical flare (new course of EEN,	We will aim to demonstrate superiority of adjunctive antibiotic therapy + SOC over SOC alone in patients with a relapse-associated microbiome. Group A1 and A2 will be compared and sustained remission is the principal analysis variable.

	need to restart steroids), no steroid dependence, no biologic (anti-TNF) use, and no intestinal surgery by 12 months.	We understand that the number of patients recruited is not enough to draw conclusions regarding efficacy of the interventions. The protocol was written to meet the requirements of clear primary and secondary endpoints with a detailed analytical plan after scientific review and subsequent approval by the Institutional Review Board of the University of North Carolina.
Exploratory		
To evaluate the potential efficacy of personalized adjunctive antibiotic therapy in improving PRO, components of established disease activity measures in remission, as well as 'biochemical' remission in pediatric subjects undergoing SOC induction therapy for mild to moderate Crohn's disease who have a relapse-associated microbiome profile	Longitudinal (clustered and with respect to baseline) changes in disease activity indices (PCDAI) components, and inflammatory markers in blood and stool: PCDAI score (0-100) Normalization of CRP (mg/dL) Fecal calprotectin (FCP) (microgram/g) Longitudinal (clustered and with respect to baseline) changes PRO: IMPACT (self-report, ages 9-17 years)	Multiple secondary variables are needed to describe clinically relevant treatment benefits. Depending on the results of the microbiome analysis, either groups A2 and B will be pooled and compared with group A1 or groups A1 and B will be pooled and compared with group A2 (section 9.4.3).
To investigate relationship between changes in subject microbiome composition and changes in disease activity over time.	Longitudinal (clustered and with respect to baseline) changes in fecal microbiome taxonomic composition or in total gene (metagenome) content. The changes will be analyzed for association with changes in disease activity (e.g. relapse or sustained remission) over time.	These changes are exploratory and not intended to demonstrate clinical benefit of treatment

Design

This is a 52-week, multicenter, randomized, controlled, open-label add-on design clinical trial pilot trial to test the feasibility of a larger trial evaluating the efficacy of adjuvant antibiotic therapy in pediatric patients with mild to moderate CD. This study will not be blinded.

A total of 20 subjects between the ages of 3 and 17 years inclusive will be enrolled in eight investigational sites located in Canada, the United States, the Netherlands and Israel.

For a full description of the study design see section 4.1 and 4.2 of this protocol.

Analyses

Feasibility will be assessed by the proportion (relative to the number of enrolled patients at visit 2 - week 0) of patients that are successfully randomized in randomization procedure following microbiome analysis, proportions of patients per treatment arm, proportion of subjects that complete 1 year endpoint.

Description of analysis is described in section 9.4.

Development of full scale trial

If results from this pilot trial show adequate feasibility of the protocol, there will be no substantial changes to the protocol before resubmitting this protocol for approval for a full scale trial. If this will be the case, then data of subjects included in the pilot trial can also be included in the full scale trial.

If results from subject's baseline stool sample are not available at randomization (week4), we will have to redesign the randomization timepoint. Also, if proportions of subjects within treatment arms vary strongly from expected, we will have to redesign the study procedure.

Additional remarks

This protocol was approved by IWK Health Centre (Halifax, Canada), North Carolina Children's Hospital (Chapel Hill, North Carolina, USA), Wolfson Medical Centre (Tel Aviv, Israel), Sheba Medical Centre (Tel Aviv, Israel). The FDA (USA), Health Canada and Ministry of Health (Israel) have reviewed the protocol and approved it.

The scientific review board of the University of North Carolina requested changes in the protocol to be made based on a full scale trial (endpoints, power analysis, statistical analysis).

SPECIFIC INFORMATION FOR THE NETHERLANDS

Study procedures

A window of study timepoint +/- 5 days is allowed for all visits/questionnaires/sampling.

Data sharing and storage

Data of participants will be stored until 25 years after the end of the study. Adequate and accurate records of each study visit will be maintained to enable the conduct of the study to be fully documented.

Paperwork as needed in the trial master file (TMF) concerning the participants are stored in a locked office at the Amsterdam University Medical Centre (AUMC), location AMC. A database with coded information and examination results will be maintained and stored on the research server of the Amsterdam University Medical Centre. The key for identifying the data is saved on a different server at the Amsterdam University Medical Centre.

Only pseudonymised information and material will be shared between study sites and the AMC and/or other centers where patient material will be analysed within the study.

Recruitment and Informed consent

Participants attending the outpatient clinic of the Amsterdam UMC, location AMC and VUmc, whom are suspected of or have been diagnosed with CD will be eligible to participate in this study. The treating physician will inform parents and children about the study when they visit the outpatient clinic of the Amsterdam UMC. A member of the research team will provide more detailed information about the study and will give the information leaflet and informed consent form. Children and parents are then asked for their signed informed consent in order to be able to participate in the study. Informed consent will be obtained from the participant (depending on the participants age) and their legal representatives conform WGBO article 7. After counseling, informed consent (a copy of the signed informed consent form is given to the participants and this is noted in the file) is signed by both children and parents (if child is 12 – 15 years old). In daily practice, the subject is often joined by one parent. The other parent is allowed to sign at home before or after the visit. As long as both signatures are collected, it is acceptable. In situations where only one parent/legal guardian is known or if one parent is deceased, the absence of the second signature is explained on a Note-to-File. A copy of the signed informed consent form is given to the participant.

The local investigator of each participating centre will maintain a list of appropriately qualified persons to whom he/she has delegated significant trial-related tasks.

Objection by minors or incapacitated subjects

This study will follow the conduct code concerning resistance in minors who participate in clinical trials as defined by the Dutch Paediatric Society. Informed consent will be asked from parents/guardians of children < 12 years. In children/adolescents >= 12 years informed consent will be asked from the parents-guardians and the children/adolescents.

Medicinal products

In the AmsterdamUMC, preparation, packaging and labeling of medicinal products will be done by the hospital's Trial Pharmacy. The choice of medicinal products will be: Metronidazole 500 mg, Metronidazole 250 mg, Azitromycin 250 mg.