

BMJ Open Personalised azithromycin+metronidazole (PAZAZ), in combination with standard induction therapy, to achieve a faecal microbiome community structure and metagenome changes associated with sustained remission in paediatric Crohn's disease (CD): protocol of a pilot study

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ABSTRACT

Introduction Early relapse in Crohn's disease (CD) is associated with a more severe disease course. The microbiome plays a crucial role, yet strategies targeting the microbiome are underrepresented in current guidelines. We hypothesise that early manipulation of the microbiome will improve clinical response to standard-of-care (SOC) induction therapy in patients with a relapse-associated microbiome profile. We describe the protocol of a pilot study assessing feasibility of treatment allocation based on baseline faecal microbiome profiles.

Methods and analysis This is a 52-week, multicentre, randomised, controlled, open-label, add-on pilot study to test the feasibility of a larger multicentre trial evaluating the efficacy of adjuvant antibiotic therapy in 20 paediatric patients with mild-to-moderate-CD ($10 < \text{PCDAI} \leq 37.5$; PCDAI, Pediatric Crohn's Disease Activity Index). SOC induction treatment will be Crohn's Disease Exclusion Diet+Partial Enteral Nutrition (CDED+PEN). Relapse-associated microbiome signatures will be evaluated using 16S rRNA gene sequencing and a previously generated Bayesian predictive model (BioMiCo) based on baseline stool. At week 4, patients in remission with relapse-associated signatures (group A) will be randomised to CDED+antibiotics (A2) or CDED+PEN alone (A1). Patients in remission without this signature will continue CDED+PEN alone (B). Patients not in remission will receive CDED+antibiotics regardless of their microbiome signature (C). Subjects in group A2 or C will receive a combination of azithromycin 7.5 mg/kg (weeks 4–8: 5 days/week; weeks 9–12: 3 days/week) with metronidazole 20 mg/kg/day (weeks 4–12). Primary outcomes will assess feasibility of treatment allocation and possible efficacy to sustain remission ($\text{PCDAI} \leq 10$, no need for reinduction). Exploratory outcomes will include changes in PCDAI, inflammatory markers and patient-reported outcomes.

STRENGTHS AND LIMITATIONS OF THIS STUDY

- ⇒ The study design ensures that only patients who could benefit from add-on antibiotic treatment will be eligible for this intervention.
- ⇒ This is the first prospective study using a microbiome prediction model (BioMiCo) based on baseline stool sample to determine (add-on) therapy.
- ⇒ The number of pilot study participants will be too few to draw conclusions concerning efficacy, especially since the study comprises different treatment groups, but will inform power calculation for the larger trial.

We will additionally explore changes in faecal microbiome taxonomic composition between groups.

Ethics and dissemination This study was approved by METC-AMC and CCMO (Netherlands) and IWK Health Centre (Canada). The first version of this protocol was approved by North Carolina Children's Hospital (USA), Wolfson Medical Centre (Israel). The FDA (USA), Health Canada and Ministry of Health (Israel) have reviewed and approved the protocol. Results will be published in international peer-reviewed journals and summaries will be provided to the funders and participants.

Trial registration number NCT04186247.

INTRODUCTION

Crohn's disease (CD) is a major phenotype of inflammatory bowel diseases (IBD) and is characterised by relapsing and remitting episodes of inflammation in the gastrointestinal (GI) tract. IBD diagnosis occurs in up to 15% of all patients during childhood and

adolescence, at a time when disease activity can severely impact (emotional and pubertal) development, growth and quality of life.^{1–4}

The exact cause of IBD remains unclear, but the disease is hypothesised to result from a defective or inappropriate activation of the mucosal immune system in response to the gut microbiome in genetically susceptible individuals.⁵ IBD results from a complex interplay of genetic, immunologic and environmental factors that manifest through the gut microbiome.^{6,7} Dysbiosis in IBD patients has been characterised by decreased microbial diversity, decreased abundance of Firmicutes and increased Proteobacteria.^{8,9} Different animal models have confirmed the role of the microbiome in IBD by showing that inflammation does only develop in the presence of enteral bacteria and that the degree of inflammation is influenced by the bacterial load and composition within the GI tract.^{10–12}

Chronic inflammation of the GI tract caused by CD can lead to progressive tissue damage with significant complications, including abscesses, fistulae and intestinal strictures.¹³ All of these complications are characterised by transmural translocation of bacteria.^{14,15} Current treatment options for CD therefore both focus on symptom control, and also on maintaining clinical remission and mucosal healing (deep remission) for a prolonged period of time.¹⁶ Current treatment strategies like corticosteroids, thiopurines, methotrexate and biologics primarily work by modulating the immune system, with the exception of exclusive enteral nutrition (EEN) of which the exact mechanism of action remains unknown.¹⁷ Recently, Crohn's Disease Exclusion Diet+Partial Enteral Nutrition (CDED+PEN) was shown to have comparable efficacy but superior tolerance for induction and maintenance of remission compared with EEN in a randomised controlled trial in children with mild-to-moderate CD.¹⁸ However, treatment responses to all currently applied therapeutic strategies are highly unpredictable and long-term remission rates rarely exceed 50%.^{19,20}

Microbiome dysbiosis is increasingly considered a key factor in CD pathophysiology, by observation that microbiome community structure and function is strongly associated with early and sustained response to therapy and could therefore hypothetically serve as a treatment target for CD. Antibiotics, which directly target bacteria in the GI tract, could therefore possibly obtain a more prominent role in CD therapy.²¹ However, while several meta-analyses support the use of antibiotics in controlling luminal inflammation, results of individual trials are heterogeneous and data in children are particularly sparse.^{22,23} Recently, a combination of azithromycin+metronidazole has been found to be superior to metronidazole alone for induction of remission in children with mild to moderate CD.²⁴ 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 a specific microbiota signature involved in a subgroup of CD patients.

Current treatment strategies have previously been shown to affect the microbiome in different ways.^{25–27} Microbiome data from the CDED-RCT showed that a baseline faecal microbiome signature with predominance of Proteobacteria did not fully correct with nutritional therapy by week 12 and is associated with recurrence of disease.^{18,28} Studies characterising the microbiome have found distinct microbiome profiles to be associated with different clinical outcomes or responses to treatment.^{25,29,30} Incorporating these specific microbiome profiles in prediction models have not led to any change in clinical practice to date.^{30–32} We have recently shown that stool microbiome communities can predict remission in treatment naïve paediatric CD patients when using Bayesian methods (AUC 0.79).³³ We found that children with higher risk for relapse in the first year show a specific Proteobacteria predominance in their stool samples, present before start of treatment.

With this knowledge, we hypothesise that the risk of early flare after achieving clinical remission by SOC nutritional induction therapy in CD patients with a relapse-associated (ie, Proteobacteria-rich) microbiome signature can be reduced by adjunctive induction therapy with oral antibiotics (azithromycin+metronidazole). The aim of the proposed pilot trial is to determine the feasibility of a multicentre trial on different continents with treatment allocation at week 4 based on baseline faecal microbiome profiles.

METHODS AND ANALYSIS

Study design

This is a 52-week, multicentre, randomised, controlled, open-label add-on design pilot study to test the feasibility of a larger trial evaluating the efficacy of adjuvant antibiotic therapy in paediatric patients with mild-to-moderate CD. The SOC induction treatment in this study will be the CDED+PEN. This study will not be blinded.

Participants

A total of 20 patients will be recruited by their treating paediatric gastroenterologist and enrolled in four academic hospitals located in The Netherlands, Canada and the USA. All patients newly diagnosed with Crohn's disease or existing diagnosis with need of re-induction will be reviewed for eligibility.

Informed consent procedure

The treating physician will inform parents and children about the study during their hospital visit. A research team member will provide more detailed information about the study, including information leaflet and informed consent form. Participants will have the opportunity to carefully review the written consent form and ask questions prior to signing. After counselling, informed consent will be signed by children and/or parents (according to applicable regulations mandated by country) and a research team member. 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.

Eligibility criteria

Inclusion criteria

1. Male or female, aged 3–17 years
2. CD diagnosis ≤ 36 months according to standard clinical and histological criteria³⁴
3. Mild-to-moderate active disease (PCDAI >10 (or >7.5 excluding height item) and ≤ 37.5).
4. Evidence of active inflammation (<30 days prior to week 0 visit) based on either:
 - a. Faecal calprotectin (FCP) ≥ 250 $\mu\text{g/g}$.
 - b. OR endoscopic and histologic evidence of inflammation obtained during endoscopy.³⁴
5. Provision of signed and dated informed consent form and stated willingness to comply with all study procedures and availability for the duration of the study

Exclusion criteria

1. Stricturing, penetrating (intestinal or perianal) and/or fistulising disease.
2. History of intestinal resection.
3. Current *Clostridioides* (formerly *Clostridium*) *difficile* infection (if tested for clinical indication).
4. Current or previous use of anti-TNF/other biological.
5. Oral aminosalicylates discontinuation/dose adjustment <14 days prior to week 0 (nb: stable doses (no change <14 days prior to week 0) of azathioprine, 6-mercaptopurine or Methotrexate (MTX) are not a reason for exclusion).
6. Intravenous anti-infective <35 days or oral anti-infectives <14 days prior to week 0.
7. Current use of cyclosporine, tacrolimus or mycophenolate mofetil.
8. Treatment with another investigational drug/intervention <30 days prior to week 0.
9. Faecal microbial transplantation <35 days prior to week 0.
10. Known allergy or intolerance to azithromycin or metronidazole.
11. Risk factors for arrhythmia including history of prolonged QTc, hypokalaemia or hypomagnesaemia, resting bradycardia or concurrent treatment with other drugs with potential for QT prolongation.
12. Prior diagnosis of any haematologic condition/blood dyscrasia which could result in leukopaenia (even if leucocyte count is normal at screening).
13. Pregnancy or lactation.
14. History of Cockayne syndrome.
15. Screening laboratory results that show any of the abnormal results depicted in [box 1](#).

Treatment groups

A baseline stool sample provided at, or within 30 days of week 0, will be processed for 16S rRNA gene sequencing via the V4–V5 region and analysed by a blinded bioinformatician at Dalhousie University, Canada. Sequencing results

Box 1 Abnormal results in screening laboratory values

1. AST, ALT $>2 \times \text{ULN}$.*
2. Urea, creatinine $>1.5 \times \text{ULN}$.*
3. White cell count $<3.0 \times 10^9/\text{L}$.
4. Total bilirubin $\geq 20 \mu\text{mol/L}$ †
5. Haemoglobin $<80 \text{g/L}$.
6. Platelets $<100\,000/\text{mL}$.

*As determined locally by each site.

†Except for subjects with isolated elevation of indirect bilirubin relating to Gilbert syndrome.

ALT, alanine transaminase; AST, aspartate transaminase.

will be available at least week 4 (at the latest) in order to ensure correct group allocation. At week 4, subjects will be assigned to group A or B based on posterior probability (PP) of having a ‘relapse-associated’ microbiome, defined as $\geq 30\%$ PP of carrying a relapse-associated microbiome signature as determined by Bayesian analysis. Subjects in remission (PCDAI ≤ 10) that have $\geq 30\%$ PP of a relapse-associated microbiome (group A) will be randomly assigned to continue CDED+PEN alone (group A1) or to receive add-on antibiotic therapy in addition to CDED+PEN (group A2) for the next 8 weeks (weeks 4–12). Subjects with $<30\%$ PP of relapse-associated microbiome (group B) will continue on CDED+PEN alone for the next 8 weeks. If patients have not achieved clinical remission (PCDAI >10) at week 4, they will be assigned to group C to receive add-on antibiotic therapy for 8 weeks, regardless of their microbiome profile. See [figure 1](#) for an overview of the study design with treatment groups and interventions.

Standard-of-care induction treatment

All subjects who enter the study will receive standard-of-care nutritional therapy for induction of remission in mild-to-moderate disease by the CDED. This is a whole-food diet coupled with PEN which was shown to be equally effective for induction of remission at week 6 with higher tolerance, including superior sustained remission up to week 12, when compared with EEN in a recent randomised controlled trial. CDED is designed to reduce exposure to dietary components that are hypothesised to have negative effects on the microbiome and intestinal barrier.^{27 28 35} The diet consists of 2 stages: CDED with 50% calories from PEN (Modulen IBD, Nestle) in weeks 0–6 (stage 1), followed by CDED with 25% PEN from weeks 7–12 (stage 2). Further details on this diet are published elsewhere.^{18 36}

Possible add-on intervention

A combination of azithromycin and metronidazole was shown to be significantly more effective than metronidazole alone for induction of remission in a randomised controlled trial in mild to moderate CD.²⁴ The combination of these antibiotics covers a wide variety of bacteria that colonise the small and large intestine. Moreover,

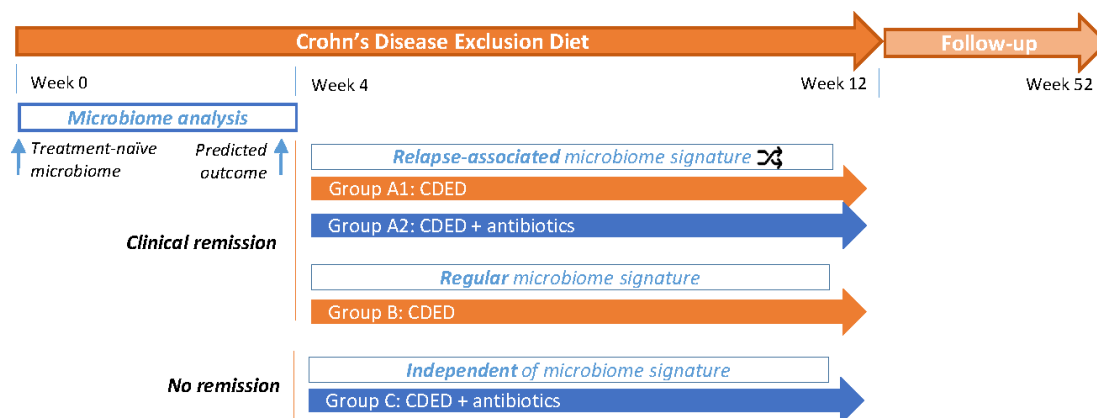


Figure 1 Overview of study design including treatment groups and possible intervention. Remission defined as PCDAI $\leq$ 10. $\curvearrowright$: randomisation. CDED, Crohn's Disease Exclusion Diet; PCDAI, Paediatric Crohn's Disease Activity Index.

azithromycin has specific features, including intracellular penetration and effect on biofilms, and can therefore target specific bacteria that are hypothesised to be critical for CD-related inflammation (like *Escherichia coli*).^{37–39}

Subjects randomised to group A2 or assigned to group C will receive a combination of oral azithromycin+metronidazole in addition to CDED+PEN in weeks 4–12. Azithromycin will be administered at a dose of 7.5 mg/kg (max. 500 mg/day) for five consecutive days per week for 4 weeks (weeks 4–8) and then three consecutive days/week (weeks 9–12). Metronidazole will be administered 10 mg/kg two times per day (20 mg/kg/day, max. 1000 mg/day) for 8 weeks (weeks 4–12). These dosages are based on a recent RCT using the same combination therapy in children with CD.²⁴ In case of intolerance, patients can be instructed to reduce the dose of metronidazole by 25% to 15 mg/kg/day and divide the frequency to three times daily at the discretion of the responsible physician.

Maintenance treatment

Start of maintenance therapy is allowed from week 4 of induction therapy onwards, consistent with recent guidelines. Subjects will be recommended to receive 15 mg/m² subcutaneous (s.c.) methotrexate (MTX) as maintenance therapy (max. 25 mg weekly), unless there are contraindications or oral MTX is preferred by treating physician and/or family.^{40 41} The use of MTX is not a condition to take part in this study and is not intended to trigger patients being excluded from or leaving the study.

Timeline and follow-up

Participants will have seven hospital visits in this study (screening, weeks 0, 4, 12, 24, 36 and 52). In case of flare, there will be an extra visit when the patient come to the hospital for regular care.

After week 12, further treatment for disease will be as per SOC—physician's choice. Participants will be followed up to week 52. See [table 1](#) for an overview of time points and procedures. All clinical (laboratory) data from study visits will be recorded anonymously in the electronic case report form (www.castoreddc.com) derived from source documents. Study data will be stored for 25 years.

Feasibility

The purpose of this pilot trial is to assess feasibility of a multicentre trial on different continents with treatment allocation at week four based on microbiota results from baseline stool samples, determined at Dalhousie University, Canada. Outcomes of this trial will provide information on the workability of this protocol and will inform power calculation for the larger trial.

Adherence

Subjects in the antibiotic treatment arms will be asked to complete a medication calendar to record the dose and any problems encountered while administering the dose. Additionally, compliance will be monitored by counting of the returned tablets at week 12 visit by study staff.

Outcomes

Primary outcome

The primary outcome of this pilot study focuses on the potential feasibility of a full-scale trial. These will be the proportion of subjects who are successfully allocated following baseline stool results, proportion of patients randomised in the randomisation procedure and the proportion of subjects who complete the 1-year endpoint.

In preparation for a larger trial, we will assess the proportion of subjects in sustained remission at 52 weeks after starting SOC induction therapy. Sustained remission will be defined as PCDAI $\leq$ 10 at week 52 and no need of reinduction for clinical flare (new course of CDED+PEN or EEN, need to start steroids), no steroid dependence, no biologic use and no intestinal surgery by 12 months.

Secondary and exploratory outcomes

Exploratory outcomes will be changes in disease activity index components (PCDAI), inflammatory markers in blood and stool (CRP normalisation (mg/dL), FCP (mcg/g)), as well as changes in patient-reported outcomes (IMPACT-III, ages 9–17) over time. We will also explore the faecal microbiome taxonomic and functional composition for association with changes in disease activity (eg, relapse or sustained remission) over time.

Table 1 Overview of time points and study procedures

Procedures	Screening (≤30 days prior to week 0)	Induction phase			Maintenance phase			Extra visit (in case of flare)
		Baseline Week 0	Possible add-on		Follow-up		End of study Week 52	
			Week 4	Week 12	Week 24	Week 36		
Informed consent	X							
Eligibility screen	X	X						
Group allocation and randomisation			X					
Intervention-related, if applicable								
Administer antibiotic			X					
Medication calendar distribution and teaching			X					
Adverse event review and evaluation			X	X	X	X	X	X
ECG†			X					
Assessments								
PCDAI score (0–100)	X	X*	X	X	X	X	X	X
Concomitant medication review	X	X	X	X	X	X	X	X
Physical examination	X	X	X	X	X	X	X	X
Stool sample (16S rRNA) for prediction of outcome at 1 year	X	X*						
Stool sample (metagenome)	X	X*	X	X	X	X	X	X
Stool sample (FCP)	X	X*	X	X	X	X	X	X
Stool sample (<i>Clostridioides difficile</i> infection testing)‡	X	X*						
Adverse event review and evaluation			X	X	X	X	X	X
Blood sample§	X	X*	X	X	X	X	X	X
IMPACT-III (ages 9 and older)		X		X	X		X	X
Urine pregnancy test¶	X		X					

Study time point window of ±5 days is allowed for all visits/questionnaires/sampling.

*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 preparation.

†All patients randomised to groups A2 and C will receive an ECG examination both prior to and at 2–4 weeks after the initiation of the study drug.

‡If performed for clinical indication, as per SOC.

§Includes haematology and clinical chemistry Albumin, C reactive protein (CRP), haematocrit (HCT), erythrocyte sedimentation rate (ESR), alanine transaminase (ALT), aspartate transaminase (AST), creatinine and bilirubin. Total blood volume collected at each visit will be 5 mL.

¶Only applicable for postmenarchal female participants initiating methotrexate.

FCP, faecal calprotectin; IMPACT-III, IBD-specific health-related quality of life questionnaire; PCDAI, Paediatric Crohn's Disease Activity Index; SOC, standard of care.

Sample size

Based on published literature, 50% of children will require repeat induction/treatment escalation by the end of their first year after diagnosis.^{42–44} Our current calculation is that 25 children carrying the high-risk microbiome signature will need to be randomised to each study arm (A1 or A2) (10% drop out assumed) in a 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 (not including lost to follow-up) 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 nor potential lost to follow-up were not used to determine the sample size for the pilot trial.

Randomisation

Subjects will be randomised centrally at the week 4 visit. Participants with a relapse-associated microbiome will be

randomly allocated into experimental treatment (A2) or control (A1) arms. To ensure 1:1 randomisation, blocks of two will be generated by using CastorEDC for patients in group A. The local study coordinator randomises the participant via CastorEDC in the presence of participant and family.

Statistical analysis

All data will be analysed according to the intention-to-treat principle. No interim analyses will be performed for this pilot study. Feasibility will be assessed by the proportion of patients who are successfully allocated at week 4 following microbiome analysis (relative to the number of enrolled patients at week 0), randomised in randomisation procedure, proportions of patients per treatment arm, proportion of subjects that complete 1 year end point. To ultimately assess sustained remission at week 52, the number, and percentage of patients with a relapse-associated gut microbiome profile will be summarised by group and the difference in sustained remission rate will be compared using a Fisher's exact test for categorical variables (comparing group A1 with A2).

For exploratory outcomes, 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. Continuous secondary outcomes will be summarised at each visit for both the observed values and the changes from baseline. A linear mixed model by group, their interaction and baseline characteristics as covariates will be used to assess the difference in means between groups at each time point. Least squares estimates for the population mean and SD will be provided at each assessment time point 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 outcome and variables will be analysed similar to the primary outcome. Missing values in continuous variables will not be imputed. Missing values in categorical variables will be imputed as non-responders.

Discontinuation of the antibiotic treatment between weeks 4 and 12 does not mean discontinuation of the study and will be moved into the group A1 (control) and receive SOC. Any patients discontinuing treatment will be reported separately.

Limitations

The number of pilot study participants will be too few to draw conclusions concerning efficacy, especially since the study comprises different treatment groups. However, to the best of our knowledge, this is the first trial with microbiome-based allocation to study groups and therefore a pilot trial is crucial to first determine feasibility. Results of this pilot study will inform power calculation for a potential larger trial.

Monitoring

Data monitoring and audits

Safety oversight will be under the direction of a Data and Safety Monitoring Board (DSMB). The DSMB will meet semiannually to assess safety and efficacy data on each arm

of the study. The DSMB will operate under the rules of an approved charter including stopping criteria that will be written and reviewed at the organisational meeting of the DSMB.

Clinical site monitoring will be conducted by an independent study monitor 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).

Each clinical site will perform internal quality management of study conduct, data and biological specimen collection, documentation and completion. Independent audits will not be conducted for this pilot trial.

Harms

The antibiotics to be used in this study are azithromycin and metronidazole. Both drugs are EMA-approved and FDA-approved, commercially available and used in paediatric practice on a daily basis.^{45 46} Moreover, additional drugs will only be given to a subgroup of children hypothesised to need additional treatment, that is, high risk for relapse (and randomised to antibiotics) and children who do not reach remission at week 4. In clinical trials, most of the reported side effects of azithromycin were mild to moderate in severity and reversible on discontinuation of the drug. Most side effects leading to discontinuation were related to the GI tract, for example, nausea, vomiting, diarrhoea or abdominal pain. Potentially serious side effects of angio-oedema and cholestatic jaundice were reported rarely. For metronidazole, the most common adverse reactions reported have been referable to the GI tract, particularly nausea, sometimes accompanied by headache, anorexia and occasionally vomiting, diarrhoea, epigastric distress, abdominal cramping and constipation. 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.

(Serious) Adverse events will be monitored throughout the whole study and reported to overseeing authorities accordingly. If there are indications that the disadvantage of participation may be greater than described in the protocol, the study will be suspended pending a further positive decision by the accredited METC.

Commencement of trial

The first patient was enrolled on 13 August 2021 in The Netherlands. There are currently 13 participants in the study.

DISCUSSION

There is a high need for novel, effective and safe treatment strategies to improve outcome of induction and

maintenance of remission in paediatric IBD. This pilot trial is the first step towards a full-scale trial, in which we propose to study personalised microbiome-targeted treatment to shift the microbiome towards a microbial signature associated with healthy state. Antibiotics are currently used in clinical practice in a wide range of IBD-complications but rarely in induction of remission. By identifying patients carrying microbiome signatures at high risk of flare and complicated disease course, microbiome-targeted therapy can be used early to improve induction of remission. If successful, in case of signs of mucosal inflammation and/or when there is a clinical disease flare, antibiotics in combination with standard dietary therapy could be preferentially used in children with a specific microbiome signature or who previously responded to these microbiome-targeted interventions. This study builds on, and directly addresses, key concerns identified in adult and paediatric cohorts, namely identifying triggers of disease development and flare, particularly impacted by diet. Reducing risks associated with uncontrolled disease as well as immunosuppressive treatment, and prioritising options with a more favourable safety profile are key targets in patient-participation efforts in the USA and Canada, both in adult and paediatric patients.^{47–49}

ETHICS AND DISSEMINATION

This study was approved by METC-AMC (2020-803) and CCMO (NL71847.018.19) (The Netherlands) and by IWK Health Centre (1023054) (Halifax, Canada). The first version of this protocol was approved by North Carolina Children's Hospital (Chapel Hill, North Carolina, USA), Wolfson Medical Centre (Tel Aviv, Israel). The FDA (USA), Health Canada and Ministry of Health (Israel) have reviewed the protocol and approved it.

All important protocol amendments will be presented to the Medical Ethics Committee of the AUMC and other participating sites and will await approval before they are implemented. All study data will be handled confidentially and coded with a unique study number. Only the research team will have access to the data. A data management plan is available.

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. 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.

Results will be published in international peer-reviewed journals and summaries will be provided to the funders of the study as well as patients and their parents/guardians

Patient and public involvement

There was no involvement of patients or the public in the design of this RCT.

PROTOCOL VERSION: 2 d.d. 9 November 2021.

Trial sponsor

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Contributors JVL is the principal investigator, designed the study, wrote the protocol, obtained funding, will contribute to bioinformatics analysis and supervised drafting of the manuscript. CMV participated in writing the protocol, coordinated the study and wrote the manuscript. KAD participated in writing the protocol, performed the bioinformatics analysis and wrote the manuscript. AO participated in writing the protocol, is a site-principal investigator and contributed to writing the manuscript. SV, WS, FS and TdM are site-principal investigators and contributed to writing the manuscript. AC and ML will contribute to the bioinformatics analysis and writing the manuscript. MH, WdJ and MAB contributed to drafting the manuscript.

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Competing interests None declared.

Patient and public involvement Patients and/or the public were not involved in the design, or conduct, or reporting or dissemination plans of this research.

Patient consent for publication Not required.

Provenance and peer review Not commissioned; externally peer reviewed.

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