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Gut microbiome predictors of advanced therapy response in Crohn's disease: protocol for the OPTIMIST prospective, longitudinal, observational pilot study in Canada

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ABSTRACT

Introduction Inflammatory bowel disease (IBD), including Crohn's disease (CD) and ulcerative colitis, is characterised by chronic and relapsing inflammation of the gastrointestinal tract, leading to significant morbidity and reduced quality of life. The global rise in IBD incidence is driven by a complex interplay of genetic, environmental, dietary and microbiome-related factors. Despite advancements in treatment, such as biologics, response rates remain variable, highlighting the need for personalised approaches. Recent research suggests that specific microbiome signatures may serve as biomarkers for predicting therapeutic efficacy, offering a potential tool for optimising treatment strategies in CD. The aim of the Optimising IBD Patient Treatment with Integrated Microbiome Investigation for Specialised Therapeutics (OPTIMIST) study is to evaluate microbiome profiles across various sample types in a Canadian CD cohort starting or already on advanced therapy, with the goal of developing predictive models for personalised therapeutics.

Methods and analysis This study is a two-phase, longitudinal, prospective observational pilot study conducted in British Columbia, Canada, involving both CD patients and non-IBD controls. Phase 1 focuses on baseline microbiome differences across participant cohorts through cross-sectional analysis. Phase 2 follows participants over 12 months to assess microbiome changes and their association with treatment response. Stool samples, intestinal biopsies from the left colon, right colon and ileum, as well as mucosal wash samples from the proximal part of the distal colon, will undergo metagenomics, metaproteomics and metabolomics analyses to explore compositional and functional differences. Data will be analysed using alpha and beta diversity metrics, differential abundance analyses and multivariate analyses to identify microbiome-based predictors of therapeutic response.

Ethics and dissemination Ethical approval was received by the Research Ethics Board (REB) of University of British Columbia-Providence Healthcare (UBC-PHC) with a REB number H23-02927. All amendments to the protocol are reported and adapted based on the requirements of the REB. The results of this study will be submitted to peer-reviewed journals and will be communicated in editorials/articles by the IBD Centre of BC and BC Children's Hospital Research Institute.

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STRENGTHS AND LIMITATIONS OF THIS STUDY

⇒ The study's comprehensive multiomics approach integrates metagenomics, metaproteomics and metabolomics, providing an in-depth analysis of the gut microbiome and its interactions with the host.
⇒ The inclusion of dietary and nutritional biomarker analyses in a longitudinal framework distinguishes this study as the first to systematically evaluate how diet influences microbiome composition and therapeutic response over time.
⇒ As a pilot study, the sample size is relatively small; however, the results will be crucial in guiding power calculations for a larger study.
⇒ While self-reported dietary data introduces potential inaccuracies, the use of a validated dietary assessment tool and blood-based nutritional analyses aims to minimise this limitation by providing a more accurate depiction of dietary intake and its impact on the microbiome.

INTRODUCTION

Inflammatory bowel disease (IBD), encompassing Crohn's disease (CD) and ulcerative colitis, involves chronic and relapsing inflammation of the gastrointestinal (GI) tract, leading to significant morbidity and reduced quality of life.[1] The incidence of IBD is increasing globally, with its aetiology involving a combination of genetic predisposition, environmental and dietary factors, and the complex interaction between the host immune response and the gut microbiome.[1]

Dysbiosis or alterations in the gut microbiome have been observed in patients with IBD compared with healthy controls.[2]

Despite advancements in treatment, particularly with biologics, clinical response rates vary significantly among patients. Approximately 30%–40% of patients fail to respond to initial biologic therapy,[3] and among responders, up to 50% may lose response over time.[4 5] This variability underscores the need for personalised treatment strategies. Recent research, including our systematic review,[6] highlights the potential of microbiome signatures as biomarkers for predicting therapeutic efficacy, providing a tool for optimising treatment response.

Emerging studies have also emphasised the crucial role of diet in modulating the gut microbiome, which in turn influences disease progression and treatment outcomes in IBD. Research investigating dietary patterns has demonstrated that certain diets can lead to alterations in microbial composition, significantly affecting inflammation and therapeutic response in IBD patients.[7] For instance, diets rich in plant-based fibres have been shown to increase the abundance of beneficial bacteria such as *Faecalibacterium prausnitzii*, which is known for its anti-inflammatory properties in the gut.[8] These findings reinforce the importance of integrating dietary assessments alongside microbiome analyses to better understand how diet–microbiome interactions contribute to disease pathogenesis and remission.

DNA sequencing and multiomics have dramatically advanced research into the gut microbiome, revealing that patients with IBD have 25% fewer taxa compared with healthy controls,[9 10] with bacterial communities often favouring a proinflammatory state.[2] Typically, patients with IBD exhibit reduced biodiversity, characterised by a decrease in Bacillota (previously Firmicutes; *F. prausnitzii* and *Roseburia hominis*) and an increase in Enterobacteriaceae.[5] Microbiota profiles also differ according to IBD subtype and disease phenotype.[9 11 12] Changes in microbial composition have been observed during both active and quiescent disease states,[9 11–14] with individual alterations being associated with future disease flare.[15 16] Several studies have shown normalisation of stool microbiota towards that of healthy controls after biologic treatment, even after adjusting for baseline dysbiosis and antibiotic use.[4 17 18]

Microbial profiles are also associated with prognostic outcomes in CD.[19 20] Various studies have investigated the association between the microbiome and disease activity or response to therapy, particularly in CD, in the hope that these associations may serve as biomarkers to predict therapeutic response.[4 11 17 18 20] These studies are mostly small and involve investigations of responses to antitumour necrosis factor (TNF) therapy, and none have focused on adult patients with CD living in Canada. Additionally, very few studies have investigated the mycobiome,[4 5] fungi residing in the GI tract or samples collected from various locations within and along the GI tract.

Research into the gut microbiome poses several challenges. A recent systematic review evaluating the microbiome in IBD and non-IBD participants was unable to draw definitive conclusions due to data heterogeneity.[21] The studies employed various methodologies and types of microbial analyses, with differences in the types of samples analysed. Less than half of the included studies reported on microbiome results in quiescent disease, and the most striking observation was the lack of consistency in results between studies.[21] The timing of stool sampling around colonoscopy is also affected by bowel preparation, with the microbiome reverting to baseline 2 weeks after colonoscopy.[22] A large international longitudinal cohort study found geographic location to be a major determinant of microbiome variance. However, the majority of variation in the microbiome was not accounted for, demonstrating the challenges of using the microbiome as a diagnostic or prognostic tool at an individual level.[23]

Given these uncertainties, we aim to evaluate microbiome profiles (bacteriome and mycobiome) across three different sample types (stool, mucosal washes and intestinal biopsies) in a cohort of adult patients with CD living in British Columbia, Canada. Specifically, we will investigate whether a microbial signature can predict response to IBD advanced therapies (ie, biologics and small molecules).

The results of this study will define the number of participants needed for a larger study. This future multicentre study will further investigate the accuracy of microbiome-based predictive algorithms for therapeutic responses in CD patients. The findings from the Optimising IBD Patient Treatment with Integrated Microbiome Investigation for Specialised Therapeutics (OPTIMIST) study will support this larger scale investigation, aiming to validate and refine predictive models across diverse populations and clinical settings. This will significantly enhance our understanding of microbiome–host interactions and the potential for personalised treatment strategies in IBD.

METHODS AND ANALYSIS

Study design

The OPTIMIST study is a two-phase, longitudinal, prospective observational pilot study (figure 1) conducted in British Columbia, Canada. Phase 1 is a cross-sectional study evaluating microbiome profiles in CD patients (experiencing active and quiescent disease) and non-IBD controls to establish baseline microbiome differences. Phase 2 is a longitudinal observational study with a 12 month follow-up, assessing changes in microbiome profiles and their association with treatment responses, validating the predictive capacity of identified microbiome signatures. The start and estimated end dates for OPTIMIST are from July 2024 to July 2028.

Study population

This study will recruit adult patients, aged 19–80 years, diagnosed with CD involving the distal small bowel

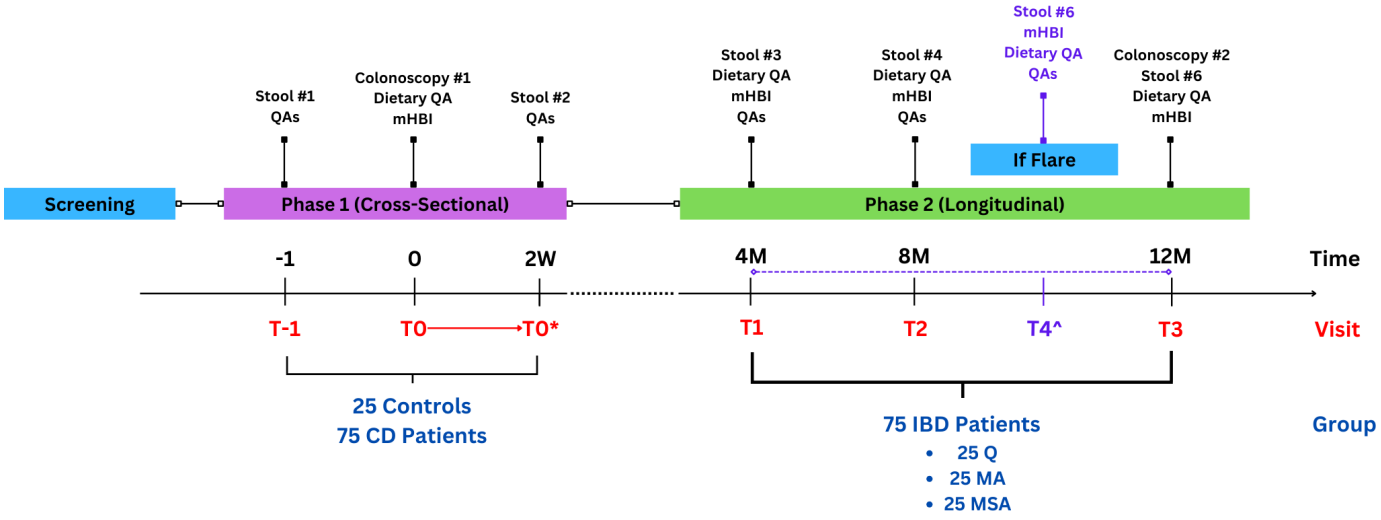

Figure 1 Schematic representation of the OPTIMIST study design. Phase 1 (cross-sectional) involves 25 non-IBD controls and 75 CD participants categorised as quiescent (Q), mildly active (MA) and moderately-severely active (MSA). At T0, participants will undergo a colonoscopy during which biopsies, mucosal washes, blood samples and disease scores (mHBI and SES-CD) will be collected. Stool samples will be provided a few days before the colonoscopy (T−1) and again 2 weeks afterward (T0), accompanied by dietary, participant, and clinical data collected through REDCap. Phase 2 (longitudinal) follows the 75 CD participants at 4, 8 and 12 months post colonoscopy (T1, T2 and T3) and, if applicable (IA), during a disease flare (T4). Participants undergoing T4 will be removed from the study and thus not partake in any further timepoints. Stool samples, mHBI scores, participant questionnaires and dietary assessments will be collected at each follow-up, with additional blood, biopsies and mucosal washes during follow-up colonoscopies at T3 or T4. *Signifies T0 stool collection, and questionnaires are received 2 weeks after colonoscopy. ^Signifies T4 collection only occurs if applicable. W, week; M, month; CD, Crohn's disease; IBD, inflammatory bowel disease; mHBI, modified Harvey Bradshaw Index; SES-CD, Simple Endoscopic Score for Crohn's Disease; REDCap, Research Electronic Data Capture; QA, questionnaire.

and/or colon, as well as sex- and age-matched non-IBD controls from the IBD Centre of BC, St Paul's Hospital, GI Research Institute Foundation (GIRI) and Pacific Gastroenterology Associates (PGA) in Vancouver, British Columbia, Canada.

Eligibility criteria

To be included in the study, participants may have either active or quiescent CD. The inclusion criteria for CD patients encompass those with varying disease states, categorised by their Simple Endoscopic Score for Crohn's Disease (SES-CD), a subjective tool completed by the gastroenterologist to assess disease severity.[24] Non-IBD controls, comprising healthy adults with no chronic conditions aged 19–80 years, will be included if they are undergoing colonoscopy as part of routine colorectal screening.

Exclusion criteria for CD patients include those with active perianal CD, defined as collection on MRI or clinically active fistula (ie, draining fistula), and those with isolated proximal small bowel or isolated upper GI CD, which are not endoscopically assessable by colonoscopy. Additionally, patients who have taken antibiotics in the last 2 months for any indication will not be eligible. Other exclusion criteria include having experienced gastroenteritis or travel outside of Canada and the USA in the last month, a diagnosis of colorectal cancer, high-grade dysplasia or a polyp ≥2 cm at baseline endoscopy, being pregnant or breastfeeding, having undergone bowel resection within the preceding 4 months, or having primary sclerosing cholangitis.

For non-IBD controls, exclusion criteria will include those found to have inflammation as deemed by the endoscopist at colonoscopy, a history of IBD in a first-degree relative, having any chronic conditions (ie, IBS, diabetes, multiple sclerosis, etc) and those who have taken antibiotics in the last 2 months. Further exclusions apply to those who have experienced gastroenteritis or travelled outside of Canada and the USA in the last month, a diagnosis of colorectal cancer, are pregnant or breastfeeding, or have had previous bowel surgeries.

Study groups

Participants will be initially assigned to groups based on their SES-CD score assessed during their baseline colonoscopy. This score will help estimate disease activity and ensure appropriate group placement.

We aim to recruit 100 participants, distributed as follows:

- *Group 1*: 25 CD patients in endoscopic remission (SES-CD score<3).
- *Group 2*: 25 CD patients with mild endoscopically active disease (SES-CD score of 3–6, or 3 with isolated ileal CD).
- *Group 3*: 25 CD with moderate to severe endoscopically active disease (SES-CD score ≥7 or ≥4 for isolated ileal CD)

- *Group 4*: 25 age-matched and sex-matched non-IBD controls.

The 25 non-IBD control participants will only take part in phase 1 of the study, while the 75 CD participants may opt to participate in either phase 1 alone or both phases 1 and 2. We are predicting a dropout rate of up to 25% therefore 20–25 participants per group are expected to complete the study.

Primary and secondary outcomes

- *Primary outcome*: compare results of microbial analyses (including bacteriome and mycobiome) across three different sample types: intestinal washing, biopsies taken during colonoscopy and stool samples.
- *Secondary outcomes*: correlation of microbiome profiles with disease activity, therapeutic response, flare rates and quality of life measures. Additional analyses will focus on the impact of dietary patterns on microbiome composition and function, and therapeutic response.

Study procedures

Participant recruitment and consent

To enrol participants in the study, patients from the IBD Centre of BC are contacted by a research coordinator via email if they meet the following criteria: (1) they are scheduled for a routine colonoscopy, which may be for disease activity assessment, dysplasia surveillance or other clinical reasons in CD participants, or for colorectal cancer screening in non-IBD control participants; (2) they appear eligible based on their medical records and (3) they have not explicitly declined participation in research studies. If patients express interest in the study, a call with the research coordinator is arranged to explain the study's objectives, procedures, risks and benefits and to verify their eligibility. Participants may then provide their consent either online (REDCap e-consent) or by paper after all their questions have been fully addressed (online supplemental files 1; 2). Non-IBD controls are only eligible to participate in phase 1 of the study, while CD participants may consent to participate in both phase 1 and phase 2.

Potential participants may also be approached by the research coordinator on the day of their colonoscopy if their appointment was scheduled on short notice. In this specific situation, the stool sample (T−1) scheduled to be collected the week leading up to the colonoscopy will not be taken.

Participant screening

After signing the consent form, participants will be asked to complete the Participant Screening Questionnaire (REDCap survey or paper version) to document their eligibility.

Study forms and questionnaires

The different study forms and questionnaires and when they are completed are summarised in table 1.

Table 1 Procedures, sample collection, and assessments for the OPTIMIST study across different timepoints (T−1 to T4)

Screening and consent						
Timepoint	**T−1**	**T0**	**T1**	**T2**	**T3**	**T4**
Screening Questionnaire (patient) (T−1 *OR* T0)	✓	✓				
Participant Questionnaire (patient)		✓				
Clinical Screening Questionnaire (research team) (T−1 *OR* T0)	✓	✓				
Consent (T−1 *OR* T0)	✓	✓				
Clinical and dietary assessments						
	T−1	**T0**	**T1**	**T2**	**T3**	**T4**
Dietary Questionnaire*		✓	✓	✓	✓	✓
Modified Harvey Bradshaw Index† (mHBI)		✓	✓	✓	✓	✓
Sample collection and tests						
	T−1	**T0**	**T1**	**T2**	**T3**	**T4**
Stool	✓	✓	✓	✓	✓	✓
Blood		✓			✓	✓
Colonoscopy SES-CD		✓			✓	✓
Mucosal washes*		✓			✓	✓
Biopsies*		✓			✓	✓
Stool tests						
Procedure	**T−1**	**T0**	**T1**	**T2**	**T3**	**T4**
Faecal calprotectin‡		✓	✓	✓	✓	✓
Clostridioides difficile toxin and faecal culture if active disease~		✓	✓	✓	✓	✓
Faecal multiomics analysis* (T−1 *AND/OR* T0)	✓	✓	✓	✓	✓	✓

The table outlines the specific procedures including screening and consent, clinical and dietary assessments, stool tests and sample collection.
*Tasks undertaken or obtained for research purposes.
†Signifies procedures completed by the Research Coordinator with assistance from the Gastroenterologist.
‡Signifies procedures that are part of routine care.

Research coordinator IBD clinical screening questionnaire

The research coordinator IBD clinical screening questionnaire collects information to assess a CD

patients' eligibility to participate in the OPTIMIST study. This includes details on disease classification, previous and current IBD therapies, disease activity scores and any IBD-related surgeries. It also gathers information on whether participants are undergoing colonoscopy as part of routine clinical care, and the presence of any GI conditions that may affect study eligibility.

Research coordinator non-IBD clinical screening questionnaire

This questionnaire, which is completed by the research coordinator, collects information to assess potential non-IBD controls' eligibility to participate in the OPTIMIST study. This includes the participant's GI history, including previous surgeries and current medications. It also verifies whether the participant is undergoing colonoscopy as part of colorectal cancer screening, which is a key inclusion criterion for non-IBD controls.

Participant Screening Questionnaire

The participant screening questionnaire gathers essential information to further assess study eligibility. It collects demographic details such as age and sex, and gender and examines lifestyle factors including exercise habits and smoking. The medical history is reviewed with a focus on conditions or recent events like antibiotic use, gastroenteritis and recent travel that could affect study eligibility. The questionnaire also screens for exclusion criteria, such as pregnancy or recent GI issues, to ensure that participants meet the specific requirements for inclusion in the study.

Participant questionnaire

The participant questionnaire collects anthropometric information, including weight and height and tracks recent weight changes, which are important for assessing nutritional status. Dietary intake over the past month is examined, alongside any menstrual-related symptom fluctuations in female-identifying participants. The questionnaire asks about a participant's past antibiotic exposure, a key factor in gut microbiome alterations, and dietary habits specifically the avoidance of certain food groups during both active disease and remission, as applicable. Additionally, it captures flare management strategies and their perceived effectiveness. A significant portion of the questionnaire is dedicated to assessing the impact of CD on quality of life through the IBDQ, which measures symptom severity, emotional well-being and the disease's effect on daily activities.

Phone call assessment form

During a scheduled phone call, participants will be assessed by a trained research coordinator to evaluate CD activity. Additionally, participants will be asked about any new or ongoing symptoms and about any recent antibiotic or probiotic, prebiotic or postbiotic use to monitor any potential influences on the gut microbiome.

IBD advanced therapy form

The advanced therapy form will be used to document any changes in the participant's advanced therapy regimen. This form collects data on whether a new advanced therapy has been initiated or if there has been a dose escalation or reduction of the current therapy. It records the names of the previous and new therapies, along with the start dates and dosages. This form, completed by the research coordinator via chart review, is crucial for tracking treatment modifications over time, which will be correlated with microbiome and clinical data to assess the impact of these changes on treatment outcomes.

Dietary data

Participants will record their dietary intake using a 3 day dietary assessment tool, including 2 weekdays and 1 weekend day, to capture variability in diet across the week, at most time points (T0, T1, T2, T3 and T4 (if indicated)). The dietary information collected will be used to determine each participant's energy, macronutrient and micronutrient intake over time. Investigating how dietary components and patterns affect gut microbiota composition and function in CD patients is crucial, as diet has been shown to be a key modulating factor of the gut microbiota.[17 23]

Clinical data

Clinical data, including disease activity scores, will be collected at T0, T1, T2, T3 and T4 (if indicated) using the modified Harvey Bradshaw Index (mHBI), a tool specifically adapted to provide a comprehensive evaluation of CD severity by assessing general well-being, abdominal pain, stool frequency, abdominal mass and extraintestinal symptoms.[25] Additionally, the SES-CD, which measures disease severity based on endoscopic findings, will be scored by the gastroenterologist at T0, T3 and T4 (if indicated) to monitor disease progression and therapy response over time.

Defining disease flare

In the OPTIMIST study, a CD flare is considered to occur if the following criteria are met:

1. Clinical symptoms: a participant's mHBI score reaches 5 or higher, where a higher score indicates worsening disease severity. Additionally, there must be an increase of 3 or more points in the mHBI score from the participant's baseline score, indicating a significant worsening of symptoms.
2. Biochemical evidence: there may be supporting biochemical evidence of increased disease activity. This can include a rise in C-reactive protein levels to 5 µg per litre of blood or higher, or an increase in faecal calprotectin levels to 250 µg per gram of stool or more. These markers indicate increased inflammation in the body or gut.
3. Endoscopic confirmation: CD flare is further confirmed through a follow-up colonoscopy that shows active disease. Specifically, this is demonstrated by an

SES-CD score greater than 7, or greater than 4 in cases of isolated ileal CD.

Phase 1: cross-sectional study for non-IBD control and CD participants

Consented CD and non-IBD control participants will be provided with a stool sample collection kit (along with verbal, written and pictorial instructions) to collect a stool sample (T−1) within the week before their colonoscopy (first bowel motion of the day), and their treating gastroenterologist will be notified of their participation in the study through their electronic medical records. Participants who have been approached on the day of their colonoscopy will not provide the T−1 stool sample but will follow the rest of the study protocol.

For all participants, blood (20–25 mL), mucosal wash (20 mL) and intestinal biopsy (9–15 samples) will be collected by gastroenterologists during the colonoscopy procedure (T0). The participant's mHBI score will be assessed on the day of the colonoscopy based on symptoms experienced the previous day.

A week and a half after the colonoscopy, the participants will be asked to report their dietary intake for 3 days (2 weekdays and 1 weekend day) using a validated dietary assessment tool. Additionally, non-IBD control and CD participants will be asked to fill out a participant questionnaire, and CD participants will also fill out the Inflammatory Bowel Disease Questionnaire (IBDQ).[26] Approximately 2 weeks after the colonoscopy, the participants will be asked to collect a stool sample (T0) using the stool sample collection kit provided on the day of their colonoscopy.

Phase 2: longitudinal study for CD participants

Following baseline assessments, non-IBD control participants will not require any additional follow-up but will be contacted at 12 months to check for any new diagnoses.

The CD participants who have consented to participate in phase 2 will provide the following samples and data at 4, 8 and 12 months post colonoscopy (T1, T2 and T3, respectively): stool sample, 3 day dietary intake information, clinical score (mHBI) and information on the use of antibiotics, probiotics, prebiotics and postbiotics in the past 4 months. Additionally, any changes in advanced therapy regimens (dose or drug) will be reported.

If a follow-up colonoscopy is required 9–12 months after the initial colonoscopy as part of routine care, blood samples, mucosal wash and biopsy samples (T3) will be collected. During each colonoscopy, mHBI and SES-CD scores will be calculated. If a disease flare occurs during phase 2 and necessitates an additional colonoscopy within the 12 months following the initial procedure, the same samples listed above will be collected (T4). Participants who experience a flare during phase 2 will not partake in any remaining timepoints they have not yet reached and will be considered to have completed the study.

Sample collection and data acquisition

Study timepoints

Data and sample collection will occur at specific time points throughout the study to capture comprehensive information on each participants' microbiome, clinical status and dietary intake. The designated time points are as follows:

- *T−1*: this initial time point occurs within the week leading up to the baseline colonoscopy. During this time, participants will complete the participant screening questionnaire, the study team will complete the clinical screening questionnaire and participants will collect a stool sample for faecal calprotectin and multiomics analysis.
- *T0*: this refers to the day of the baseline colonoscopy and the following 2 weeks. During this period, participants will provide blood samples and undergo the colonoscopy procedure, during which mucosal washings and intestinal biopsies will be collected. The mHBI will be completed with assistance from the gastroenterologist to assess the participants' clinical status, and the SES-CD score will be calculated by the gastroenterologist. Participants will also complete the participant questionnaire, which includes the IBDQ. Additionally, dietary intake information and stool samples will be collected approximately 2 weeks after the procedure.
- *T1 (4 months), T2 (8 months), T3 (12 months)*: at each of these time points, participants will collect stool samples and will complete the 3 day dietary assessment. The mHBI will also be reassessed during each visit to monitor changes in clinical status since the baseline visit. At T3 (9–12 months), participants will undergo a follow-up colonoscopy, if medically required, where additional mucosal washings and intestinal biopsies will be collected. These combined data collection points allow for a longitudinal, comprehensive understanding of how the microbiome and clinical condition change over time in response to advanced therapies.
- *T4*: should a CD patient experience a disease flare at any point during phase 2, additional data and samples will be collected. This includes a stool sample, completion of the 3 day dietary assessment and reassessment of the mHBI. An additional colonoscopy will also be performed, during which blood, mucosal washings and intestinal biopsies will be collected. These flare-related data and samples are collected at T4 to provide insights into the microbiome's response to disease exacerbation and assess the predictive value of microbial changes. After T4, participants will not be required to provide further study data, and their participation in the study will be considered complete.

Sample collection and analysis

Stool samples

Stool samples will be collected at all timepoints: T−1, T0, T1, T2 and T3 or T4 (if indicated). Participants will

collect stool samples from their first bowel movement of the day using standardised collection kits provided by the study team. Each kit includes detailed instructions to ensure proper and hygienic sample collection. The kits also contain an anaerobic sachet to maintain low-oxygen conditions, preserving the viability of anaerobic microorganisms during transit. After collection, samples are sealed and shipped fresh the same day using overnight courier services, ensuring that the sample arrives at the laboratory within 24 hours to minimise potential degradation or changes in microbial composition.

On arrival at the laboratory, stool samples are immediately aliquoted in a biosafety cabinet. The processing involves collecting material from the outer layer of the stool first, followed by anaerobic in-vitro culturing analysis. The rest of the sample is homogenised, and aliquots are taken for inner layer and multiomics analysis (ie, metabolomics, metaproteomics). Stool aliquots are then stored at −80°C until future analysis.

Microbial DNA from stool samples will be extracted using a commercial kit optimised for high yield and integrity, and the DNA quality is assessed via PicoGreen dsDNA assay and gel electrophoresis. Quantitative PCR is performed to quantify total bacterial and fungal concentrations. Shotgun metagenomics and internal transcribed spacer (ITS) sequencing will be employed to analyse bacterial and fungal components of the microbiota. This approach is critical as previous research has shown that microbial diversity and specific taxa are associated with disease activity and response to treatment.[27] Metaproteomics and metabolomics will further examine microbial proteins, functional pathways and metabolites, providing insights into the functional interactions within the gut ecosystem. Additionally, stool samples will be used for anaerobic culturing to isolate and study key microbes under conditions that mimic the gut environment. Finally, faecal calprotectin analysis, which will be done as part of routine care, will provide data on levels of inflammation in the GI tract. Stool samples will be stored at −80°C for future processing/analysis.

Intestinal biopsy samples

Intestinal biopsy samples will be obtained during colonoscopy (T0 and T3 or T4 (if indicated)) from three intestinal sites: ileum, right colon and left colon. Both inflamed and non-inflamed regions will be sampled to capture site-specific microbiome variations. Up to 15 biopsies will be collected per patient, depending on the type of consent provided by the participant. Negative control samples will also be collected to identify and minimise potential contamination during the procedure. These controls include swabbing key surfaces such as the gastroenterologist's personal protective equipment and endoscopy instruments. Collecting these negative controls is essential for ensuring the accuracy and reliability of microbiome analyses, particularly when working with low-biomass samples.[28] Once collected, biopsy and control samples will be stored at −80°C until future processing and analysis. Additionally, organoids will be generated from a subset of biopsy samples to study epithelial-microbiome interactions and mucin production, and RNA sequencing will be employed to profile gene expression and identify disease markers.[29 30]

DNA will be extracted from biopsy samples using the same optimised commercial kit as for stool samples, ensuring high yield and integrity. Extracted DNA will undergo quality assessment using PicoGreen dsDNA assay and gel electrophoresis to verify DNA concentration and integrity. Quantitative PCR will be performed to quantify bacterial and fungal concentrations, with positive control spikes included to validate extraction and amplification efficiency. Biopsy samples will also be analysed using shotgun metagenomic and ITS sequencing, metaproteomics and metabolomics to explore the functional pathways, microbial proteins and metabolites present, thereby providing a comprehensive view of the interactions between the microbiome and host.

Mucosal wash samples

Mucosal wash samples will be collected during colonoscopy from the proximal portion of the distal colon at T0 and T3 or T4 (if indicated). To collect the sample, 60 mL of sterile saline will be flushed through the working channel of the colonoscope to irrigate the mucosal surface. After a few seconds, the fluid is suctioned back into a sterile trap, aiming to collect 20–30 mL of the wash. DNA will be extracted from mucosal wash samples using the same optimised commercial kit, with PicoGreen dsDNA assay and gel electrophoresis employed to assess DNA concentration and integrity. Quantitative PCR will also be performed to quantify bacterial and fungal concentrations, ensuring high-quality data for downstream analyses.

These samples will undergo shotgun metagenomic and ITS sequencing, metaproteomic and metabolomic analyses to determine the functional role of the gut microbiota, including the identification of functional pathways, metabolites and host glycoproteins.[31] Mucosal wash samples will be stored at −80°C for future processing/analysis.

Blood samples

Blood samples will be collected for immune and nutritional profiling at T0 and T3 or T4 (if indicated), and will be processed immediately. Plasma, serum and blood for RNA analyses will be collected. Peripheral blood mononuclear cells (PBMCs) will also be collected, processed and analysed to investigate immune cell populations and their responses. Flow cytometry will assess these immune cell populations, providing insights into the immunological responses to advanced therapy in CD patients. Additionally, the Olink q100 proteomics platform will measure a broad array of proteins related to inflammation and immune responses. Tempus RNA tubes will be used to collect blood for RNA sequencing to analyse gene expression. Nutritional status using plasma and serum samples

will also be assessed, using high-performance liquid chromatography, focusing on key biomarkers such as vitamin D, B_{12} and iron, all of which are linked to disease activity and response to advanced therapy.[32] Blood samples will be stored at −80°C and PBMC samples will be stored in liquid nitrogen for future processing/analysis.

Data management and data safety

All participant consent forms and questionnaires will be collected electronically to ensure efficiency and secure documentation, with each participant assigned a unique ID in place of any identifiable information. Clinical and study-specific data will be collected on paper-based forms at the study site where procedures occur and stored on a secured local area network, enabling secure sharing among authorised personnel. For electronic data capture (EDC), the REDCap system, a clinical trial EDC platform, will be used. Designated study personnel will receive thorough training in REDCap and be provided with unique usernames and passwords, secured through two-factor authentication, to access the system. Data entered into REDCap will be verified for accuracy by the research coordinator before being finalised. Paper records will be securely stored in a locked cabinet within the research facility, accessible only to designated personnel. Deidentified experimental data will be stored in encrypted databases at the Lunken Lab for a minimum of 5 years and up to 15 years, ensuring compliance with data retention policies.

Sequencing data analyses

Stool, mucosal wash and intestinal biopsy samples will be analysed using shotgun metagenomic and ITS sequencing to study the gut microbiome, including bacterial and fungal components. ITS sequencing will allow for the evaluation of fungal diversity and composition across sample types. We will assess the quality of the reads by looking at factors such as the Phred quality scores (which indicate the accuracy of the reads), the number of reads per sample (sequencing depth) and the percentage of reads that map to the host and marker databases. If any samples do not meet our quality standards, they will not be further analysed. To ensure accurate analysis, any host DNA will be removed before processing the microbial DNA.

To identify and classify the microorganisms present, we will use MetaPhIAn4,[33] a tool that maps sequencing reads to species-specific genomic markers, allowing us to determine the relative abundance of different taxa at the strain level. Additionally, HUMAnN2 will be used to create functional genomic profiles, which will provide detailed information on the gene families and metabolic pathways within the microbial community.[34]

Statistical analysis: alpha-diversity metrics, including Shannon, inverse Simpson and Chao1, will be calculated to assess species richness and evenness within groups. Non-parametric tests will evaluate differences between groups. Beta-diversity metrics, such as Bray-Curtis, Jaccard and weighted and unweighted UniFrac, will measure compositional differences between groups, with clustering visualised via principal coordinate analysis. Overall microbiome differences will be tested using PERMANOVA, and false discovery rates will be controlled using the Benjamini-Hochberg method. Differential abundance will be assessed through generalised linear models (GLMs), with exploratory analysis ensuring data meet assumptions for each test.

Dietary data from 3 day dietary assessments will be exported and analysed using mixed-effects models to explore the relationship between dietary patterns and microbiome composition, adjusting for potential confounders. GLMs will also be employed to analyse clinical data, including mHBI scores and antibiotic/probiotic use, to correlate these variables with microbiome diversity, treatment response and other clinical outcomes. Additionally, PERMANOVA will be used to test for overall differences in microbiota composition between groups, accounting for dietary intake and clinical factors, providing a comprehensive analysis of how these elements interact and influence therapeutic outcomes.

Sample size calculation

As the OPTIMIST study is a pilot study, a formal sample size calculation has not been conducted. Instead, the sample size (n=100) has been chosen based on data from similar published studies in the field.[3 17 35] This sample size is intended to provide preliminary data and inform future large-scale investigations.

Ethics and dissemination

Ethical approval was received by the Research Ethics Board (REB) of University of British Columbia-Providence Health Care (UBC-PHC) with an REB number H23-02927. All amendments to the protocol are reported and adapted based on the requirements of the REB. The results of this study will be submitted to peer-reviewed journals and will be communicated in editorials/articles by the IBD Centre of BC and BC Children's Hospital Research Institute.

Data availability

At the completion of the study, deidentified microbiome analysis and dietary data will be shared with interested participants through encrypted email over a secure network. The deidentified clinical data and samples may also be made available for future research after gaining approval from the University of British Columbia's REB. Researchers requesting access to samples and/or data must submit a methodologically sound proposal and sign a Clinical Study Transfer Agreement. The agreement stipulates that the data will be used solely for the purposes outlined in the proposal and mandates secure storage of the data. Upon project completion, the data must either be destroyed or returned to the OPTIMIST research team.

Patient and public involvement

Patients are integral to the OPTIMIST study, not only as participants but also as contributors to its ongoing refinement. A select number of participants will be invited to join a patient advisory group, where they can share their experiences throughout the study, discuss their overall satisfaction and offer suggestions for future improvements. This feedback will help ensure that the study remains patient-centered and responsive to the needs of those involved. To recognise the time and commitment of participants, a gift voucher will be offered to those who complete either phase 1 or both phases 1 and 2 of the study.

DISCUSSION

The OPTIMIST study is the first in Canada to investigate the spatial variations in microbiota across stool, mucosal wash and intestinal biopsy samples, aiming to better understand their influence on therapy response in CD. While prior studies predominantly focused on stool samples to assess luminal bacteria,[10] our recent systematic review revealed critical gaps in the literature, particularly regarding the contributions of tissue-associated and mucus-associated microbes.[6] Few studies have explored these sample types,[36 37] with even fewer examining their role in therapy response across different GI tract locations. Our review identified only four studies using biopsy samples,[36–39] and none included mucus-associated microbes, underscoring the need for research on these understudied microbial communities.

Spatial heterogeneity in microbial composition significantly impacts immune response and disease progression.[40] Microbes residing closer to the intestinal epithelium, such as those in mucus and tissue, are likely more influential in modulating immune responses than luminal bacteria. The OPTIMIST study seeks to address the lack of knowledge by examining samples from different gut regions to assess how spatial variations in the microbiome impact therapeutic outcomes.

Furthermore, prior research has primarily focused on gut bacteria while overlooking dysbiotic fungi, despite their role in modulating immune responses and disease pathogenesis in IBD.[41] For instance, the fungal species *Candida albicans* is a major modulator of CD4+ T-cell responses and an inducer of both IgA and IgG antibodies.[42 43] One study also found that non-response to advanced therapy was associated with a twofold higher baseline abundance of *C. albicans*, which remained elevated post-treatment.[5] By further examining the role of dysbiotic fungi in immune response and disease progression, as well as how these effects differ across GI tract locations, the OPTIMIST study could identify potential predictive biomarkers of therapy response.

Strengths and limitations

The OPTIMIST study is novel due to its comprehensive and innovative multiomics approach. This multifaceted analysis should provide a holistic and in-depth understanding of the gut microbiome, encompassing not only bacterial communities but also the often-overlooked fungal taxa. By exploring microbial differences across various sample types and locations along the GI tract (stool, mucosal wash and intestinal biopsy), the study will offer unique insights into the spatial variations of the microbiome and their potential impact on therapeutic responses in CD. This detailed exploration of different gut regions is crucial, as it allows for the identification of site-specific microbiota that may play a significant role in immune modulation and treatment outcomes.

Another key strength of the study is the inclusion of detailed dietary assessments and nutritional biomarker analyses. Diet is a recognised modulator of the gut microbiome,[17 23] and by employing a 3 day dietary assessment at multiple time points, the study ensures the accurate and longitudinal collection of dietary data. This comprehensive dietary data, combined with multiomics analyses, will enable the study to explore how specific dietary patterns influence microbiome composition and function in CD patients. Understanding the diet–microbiome-disease axis is critical, as it may reveal new dietary interventions that could enhance therapeutic efficacy.

Furthermore, the study's focus on a Canadian cohort of CD patients addresses a significant gap in the literature, as existing microbiome and therapy response research has been conducted outside of Canada. This focus is crucial given the potential geographical differences in microbiome composition and environmental exposures, which may influence disease phenotype and treatment response.[44]

Additionally, the study's inclusion of comprehensive mycobiome analysis—a relatively understudied area in IBD research—provides novel insights into the role of fungi in disease pathogenesis and therapeutic response.[5 41] The detailed analysis of the fungal microbiome, alongside bacterial communities, offers a more complete picture of the microbial ecosystem in CD, potentially uncovering new therapeutic targets or biomarkers.[5]

The OPTIMIST study is a pilot study, so the sample size is relatively small. While the study's design is robust, the limited number of participants may affect the statistical power of all analyses, particularly when exploring the relationship between microbiome profiles, diet and treatment response. The data generated from the pilot study will help guide power calculations for a larger, multicentre study planned in the future.

Potential confounding factors, such as variations in diet, medication adherence and environmental exposures, also pose challenges in microbiome studies.[17 31 45] The OPTIMIST study aims to control for these variables, their influence on microbiome composition and therapeutic response to ensure the data generated accurately reflects the true impact of the microbiome on advance therapy response, minimises bias and enhances the reliability of our findings.

Finally, the reliance on self-reported dietary data can result in inaccuracies that may not fully capture a person's true dietary intake. Many dietary assessment methods are susceptible to reporting bias, where participants may unintentionally or intentionally misreport their intake, leading to misclassification of dietary exposures.[46] This can weaken the observed associations between diet and microbiome composition, making it challenging to identify true diet–microbiome interactions that influence treatment outcomes. However, the use of the 3 day dietary assessment tool offers an advantage, as it guides participants through the food record process, improving accuracy.[47] Additionally, conducting nutritional blood analyses will provide a more objective measure of each participants' nutritional status, further enhancing the reliability of the data.

The OPTIMIST study aims to evaluate the feasibility and utility of microbiome profiling in predicting biologic therapy responses in CD patients. Our findings will highlight the potential for microbial signatures to guide personalised treatment strategies, improving therapeutic efficacy and patient outcomes. Building on this pilot study, future research should expand on these findings to develop robust clinical tools for personalised IBD management.

FUTURE DIRECTIONS

Individualised therapy and predictive algorithms

The aim of our study is to predict individual responses to therapy via microbial signature analysis across different sample types. This will enable clinicians to tailor therapeutic interventions based on each patient's microbiome profile, potentially improving patient outcomes, reducing treatment failures, and minimising adverse effects.

Large-scale, multicentre study

Building on the pilot OPTIMIST study, future plans involve conducting a multicentre study across Canada with a larger study population. This expanded research will be essential for developing robust predictive algorithms aimed at transitioning findings from the laboratory to clinical practice. Such a comprehensive study will significantly enhance the translation of microbiome research into effective clinical practice.

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