Official Title: Dietary Study of Human Milk Oligosaccharides with *Bifidobacterium infantis* in Antibiotic-treated Healthy Adult Volunteers

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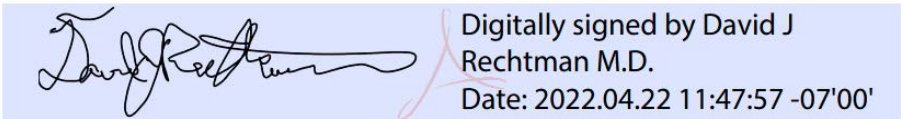
TITLE PAGE

CLINICAL STUDY PROTOCOL

PROTOCOL TITLE:	**Dietary Study of Human Milk Oligosaccharides with *Bifidobacterium infantis* in Antibiotic-treated Healthy Adult Volunteers**
PROTOCOL NUMBER:	**21-CT-003 – Revision 5**
SPONSOR:	**Prolacta Bioscience** **1800 Highland Ave.** **Duarte, CA 91010**
SPONSOR'S MEDICAL MONITOR:	**David J Rechtman, M.D.** **VP, Medical Affairs**
SPONSOR PROJECT MANAGER:	**Gregory McKenzie, Ph.D.** **VP, Product Innovation**

SIGNATURE PAGE

Author Signature / Date:



David J Rechtman, M.D. **Date**

VP, Medical Affairs

This study protocol has been reviewed and approved by the undersigned persons. It is confirmed that the information and guidance given in this protocol complies with scientific principles, the guidelines of Good Clinical Practices, the Declaration of Helsinki in the latest relevant version and the applicable legal and regulatory requirements.

Martin Lee April 22, 2022

Martin Lee, Ph.D. **Date**

VP, Clinical Research & Development

Gregory McKenzie, Ph.D **Date**

VP, Product Innovation

INVESTIGATOR PROTOCOL AGREEMENT

The signature below constitutes that I agree to the following:

- I have reviewed the protocol and the attachment(s).
- This study will be conducted according to all stipulations of the protocol, including all statements regarding confidentiality, and according to local legal and regulatory requirements and applicable United States federal regulations, International Conference on Harmonization (ICH) guidelines, Health Insurance Portability and Accountability Act (HIPAA) guidelines.
- I agree to periodic site monitoring of source documents by Prolacta Bioscience or designee and by appropriate regulatory authorities.
- I agree to supply Prolacta Bioscience with any information regarding ownership interest and financial ties with the Sponsor for the purpose of complying with regulatory requirements.

Investigator Name (Print)	**Investigator Signature**	**Date**

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Background and Hypothesis

Dietary Study of Human Milk Oligosaccharides with *Bifidobacterium infantis* in Antibiotic-treated Healthy Adult Volunteers

Background

Human milk oligosaccharides (HMO) have recently become the objects of scientific inquiry. These complex sugars are extremely heat stable and are therefore found intact in donor milk and human milk-based products following pasteurization as well as in untreated mother's own milk (Barile, 2008) (D. Barile, 2009). (M Ninonuevo, 2006). Approximately 200 different HMO structures have been identified, although any particular mother will only produce a subset of these (Ayechu-Muruzabal, 2018). Studies have shown that HMO can alter the gut microbiome by favoring the growth of certain organisms as well as, perhaps, by inhibiting others (Smilowtiz, 2014). It has also been demonstrated that HMO can act as "decoy" receptors for a variety of pathogens and toxins. (Smilowtiz, 2014). They have also been shown to directly interact with certain components of the adaptive immune system in vitro (Smilowtiz, 2014).

It is therefore reasonable to expect that these molecules may be useful as therapeutic agents or medical foods. This is particularly the case in medical settings where the HMO themselves or their metabolites may provide missing nutrients or other metabolic products for the dietary management of diseases where the microbiome plays a role, either in whole or in part. Examples include conditions as disparate as inflammatory bowel disease and stem cell transplantation.

We hypothesize that *Bifidobacterium longum* subspecies *infantis* (*B. infantis*) might be a particularly beneficial organism to expand in the microbiome when managing such diseases in combination with HMO. In infants, co-administration of *B. infantis* with human milk (via breast feeding) results in expansion of *B. infantis*, reduction in other potentially deleterious species, notably members of the Family *Enterobacteriaceae*, and acidification of baby's stool through the production of certain short chain fatty acids (Frese, 2017). These dietary effects are postulated to be due to metabolism of HMO by *B. infantis*, as infants receiving human milk in the absence of *B. infantis* don't show these effects (Frese, 2017). Importantly, *B. infantis* is not observed as a constituent of the human adult gut microbiota so it may therefore be necessary to administer both HMO and *B. infantis* to obtain the full beneficial impact of HMO in adults.

Prolacta Bioscience recently completed a study in healthy volunteers which demonstrated that a concentrate of HMO derived from human milk can drive *B. infantis* colonization in some healthy volunteers (20-CT-001, 2020). The purpose of this study, 21-CT-003, is three-fold. One goal is to determine if the rate of HMO-supported colonization with *B. infantis* is increased if the microbiome of the volunteers has been disrupted by administration of antibiotics. The second goal is to determine whether microbiome recovery post-antibiotic treatment can be improved by the presence of *B. infantis*. The third goal is to determine the mechanism of HMO-supported colonization; whether a selected subset of HMO (2'FL + LNnT) can support *B. infantis* colonization to the same extent as the full complement of HMO present in the HMO concentrate used previously.

The HMO concentrate PBCLN-010 is a frozen liquid 15% concentration of HMO derived from the permeate obtained in concentrating donor human milk during the manufacturing of Prolacta's human milk fortifiers. Previous versions of this product at 5% and 10% concentrations have been studied as a food supplement in healthy volunteers, either alone or in combination with *B. infantis.* A previous version has also been studied alone in *C. difficile* patients. No serious product-related adverse events have been reported in the course of these studies.

The synthetic HMO 2'FL + LNnT product in this trial is a powder containing 2'-fucosyllactose (2'-FL) and lactoneotetraose (LNnT). These HMO were also present in Prolacta's previous HMO studies, where donor-derived HMO concentrates were administered to subjects with no adverse events recorded. Here, they are present in a more purified mixture without the other HMO found in material used in the earlier trials. This HMO product is commercially available as a medical food in the US.

The *B. infantis* preparation being used in this trial is a dietary supplement commercially available in the United States and marketed for use in infants. *B. infantis* is characterized as "generally regarded as safe" (GRAS) (Duar, 2020).

Hypothesis

We hypothesize that the rate of *B. infantis* colonization will increase following microbiome disruption, microbiome recovery will be enhanced, and that a similar effect may be seen with use of the specific synthetic HMO.

We hypothesize that ingestion of HMO with *B. infantis* after antibiotic treatment will result in a change in immune markers as was seen previously in study 16-CLN-003 (16-CLN-003, 2016), where HMO concentrate was consumed by healthy volunteers. We will, therefore, evaluate changes in circulating cytokine and growth factor levels following the ingestion of the IP compared to baseline levels and between dose groups.

We believe that the changes in the microbiome, and possibly the changes in immune markers as a surrogate for immune activity, may be important in improving the clinical course of patients with certain diseases thought to be impacted by the microbiome.

Methods

We propose to conduct an unblinded five dosing group trial using 18 subjects per dosing group for a total of 90 healthy adult volunteers ages 18-75. The randomization scheme for this study is as follows. The first twenty subjects will be randomized into Dosing Group 1 or 2 using a randomized permuted block scheme. The next thirty four subjects will be randomized into Dosing Group 1, 2, or 3 using a similar separate randomized permuted block scheme employing a 4:4:9 ratio (group 1:group 2:group 3) in a block of 17. The remaining thirty six subjects will be randomized into Dosing Groups 4 or 5 using a separate but similar randomized permuted block approach employing a 1:1 ratio (group 4:group 5).

The IP and medications for this study include: antibiotics, HMO PBCLN-010, HMO 2'FL + LNnT, and *B. infantis*.

Antibiotics will be given to all subjects in this study: vancomycin (250 mg/dose) and metronidazole (500 mg/dose).

HMO PBCLN-010 is a frozen liquid, 15% concentration of HMO derived from the permeate obtained in concentrating donor human milk during the manufacturing of Prolacta's human milk fortifiers.

The HMO 2'FL + LNnT will be presented as individual lyophilized powder stick packs to be mixed with an appropriate diluent prior to ingestion.

B. infantis $\geq 8X10^9$ CFU will be presented as individual lyophilized powder sachets to be mixed with an appropriate diluent prior to ingestion.

The dosing regime is outlined as follows, refer to Table 1 additionally.

Subjects randomized to Dosing Group 1 will consume antibiotics three times daily (TID) for 5 days. Subjects will be followed from the first dose of the antibiotics (day 1) until day 35.

Subjects randomized to Dosing Group 2 will consume antibiotics three times daily (TID) for 5 days, HMO PBCLN-010 twice daily for 28 days and *B. infantis* once daily for 14 days. They will be followed from the first dose of the IP (day 1) until day 35.

Subjects randomized to Dosing Group 3 will consume antibiotics three times daily (TID) for 5 days and *B. infantis* once daily for 14 days. They will be followed from the first dose of the IP (day 1) until day 35.

Subjects randomized to Dosing Group 4 will consume antibiotics three times daily (TID) for 5 days, HMO PBCLN-010 twice daily for 14 days, *B. infantis* once daily for 14 days, and HMO 2'FL + LNnT twice daily for 14 days (to begin after HMO PBCLN-010 dosing has ended). They will be followed from the first dose of the IP (day 1) until day 35.

Subjects randomized to Dosing Group 5 will consume antibiotics three times daily (TID) for 5 days, *B. infantis* once daily for 14 days, and HMO 2'FL + LNnT twice daily for 28 days. They will be followed from the first dose of the IP (day 1) until day 35.

Table 1 Dosing Cohorts Regime

Dosing Group	**vancomycin** (250 mg/dose) and **metronidazole** (500 mg/dose) **(1 tablet of each antibiotic three times per day)**	***B. infantis* (1 sachet once per day)**	**Daily target=18g HMO PBCLN-010 (9g twice per day)**	**Daily target=22g HMO 2'FL + LNnT (2 sachets twice per day)**
1	D1 – D5	None	None	None
2	D1 – D5	D1 – D14	D1 – D28	None
3	D1 – D5	D1 – D14	None	None
4	D1 – D5	D1 – D14	D1 – D14	D15 – D28
5	D1 – D5	D1 – D14	None	D1 – D28

Previous versions of the HMO concentrate in this study (PBCLN-010) differing only in HMO concentration, are food supplements that have been studied in human healthy volunteers in the past with no serious adverse events reported (16-CLN-003, 2016) (20-CT-001, 2020). The synthetic HMO 2'FL + LNnT is a commercially available medical food in powder form, and the *B. infantis* is a commercially available food supplement in powder form both of which are Generally Regarded As Safe (GRAS) (Duar, 2020) (Walsh, 2020). Therefore, this study will not be conducted in the manner of a dose escalation safety study. Dosing Groups 1 and 2 will be filled first and Dosing Groups 3, 4, and 5 will be filled once Dosing Groups 1 and 2 subjects have completed the study.

Sample Size

Five dosing groups of 18 subjects, each will be included in this study for a total sample size of 90. The sample size was not determined statistically, but rather represents a typical number for an exploratory study in healthy volunteers.

Study Population

Each subject must meet all of the indicated inclusion criteria and none of the exclusion criteria noted below.

Inclusion Criteria

- Healthy adults between the ages of 18-75 years (subjects must be 18-75 at the time of consent) who can provide proof of vaccination against SARS-CoV-2. Proof may be a

physical or electronic record of vaccination or self-attestation (to include approximate vaccination date and manufacturer of vaccine) if a copy of the vaccination record is not available

- Subjects must have a BMI of 18 - 30 at screening visit
- Willingness to complete study specific questionnaires
- Willingness to complete journal to record IP dosing times, Bristol stool scores, and IP flavor questionnaires
- Willingness to complete all study procedures, clinic visits, and provide required biospecimen samples
- Willingness to collect and process stool samples at home and transport stool samples to clinic
- Sexually active females of child-bearing potential must agree to use highly effective methods of contraception during heterosexual intercourse throughout the study period and for three days following discontinuation of IP, whichever comes later. Examples of highly effective methods include the use of two forms of contraception with one being an effective barrier method (e.g., a condom and spermicide used together), or have a vasectomised partner. Abstinence is acceptable as a life-style choice. Female subjects who utilize hormonal contraceptives as one of their birth control methods must have used the same method for at least 3 months before study dosing
- Provide informed consent

Exclusion Criteria

- Subjects with a BMI of 17 or less or 31 or greater are excluded
- Women who are pregnant or breastfeeding, or intend to become pregnant during the course of this study
- Subjects who intend to take a probiotic during the study
- Subjects with self-reported diarrhea on day 1 prior to dosing, whereby diarrhea is defined as two or more episodes of watery and/or unformed stool within 24 hours
- Alcohol or drug abuse during the last 12 months, including passing a screen for drugs of abuse at screening and day 1 of the study
- Unstable medical condition, in the opinion of the investigator

- Subject with a history of allergy to vancomycin and/or metronidazole
- Clinically significant abnormal laboratory test results at screening
- Subjects who are unable or unwilling to provide stool samples on a regular basis as per study protocol
- Participation in a clinical research trial within 30 days prior to screening
- Unable to give informed consent
- Any condition which may preclude subject's ability to comply with and complete the study or may pose a risk to the health of the subject
- Known carriers of *C. difficile* prior to study start, as determined by qPCR of stool
- Known carriers of vancomycin-resistant enterococci (VRE) prior to study start, as determined by stool culture
- Subjects with history of lactose intolerance

Study Procedure

Screening Visit may occur up to and including 90 days prior (≤ 90 days prior D1) to the subject's first dose. After informed consent is obtained and subject eligibility is assessed, a physical exam will be performed, vital signs, samples of blood, stool, and urine will be obtained from subjects at screening visit (≤ 90 days prior D1). Prior to dosing on day 1, subject eligibility will be reassessed, vital signs, samples of blood, urine, and stool will be obtained from subjects.

Samples of blood, urine and stool from all subjects will be taken at screening (≤ 90 days prior D1). Blood samples will be drawn on days 1, 3, 5, 9 (+1 day), 14 (+2 days), 28 (+2 days), and 35 (+2 days). Urine samples will be taken on days 1, 3, 5, and 35 (+2 days). Stool samples will also be required on days 1, 3, 4, 5, 7 (+1 day), 9 (+1 day), 11 (+2 days), 14 (+2 days), 17 (+2 days), 21 (+2 days), 28 (+2 days) and 35 (+2 days).

Some subjects, at the discretion of the sponsor, may be asked to return to the study site and provide a stool sample on or after Day 90. These completed subjects may potentially no longer meet the Inclusion/Exclusion criteria nor conform to the dietary/medical restrictions in place for the first 35 study days. Refer to the Post Study Sample Schedule of Events for required events for these subjects.

Stool Samples

Stool samples collected at screening (≤ 90 days prior to D1) will be analyzed by qPCR to determine if the subject is a carrier of *C. difficile*. Stool samples will also be analyzed via culture for vancomycin-resistant enterococci (VRE). Subjects that are identified as *C. difficile* and/or vancomycin-resistant enterococci (VRE) carriers at the time of screening will be considered a screen fail. Screening (≤ 90 days prior to D1) stool samples collected for microbiological, metabolomic and metagenomic testing to answer questions of interest to sponsor will not be used to determine patient eligibility. Stool samples will be collected throughout the study for microbiological, metabolomic and metagenomic analyses (days 1, 3, 4, 5, 7 (+1 day), 9 (+1 day), 11 (+2 days), 14 (+2 days), 17 (+2 days), 21 (+2 days), 28 (+2 days) and 35 (+2 days). Microbiological analysis will also be conducted on the post study sample (day 90 or later) for those subjects who have been identified by the sponsor to provide a post study sample (day 90 or later).

At-home stool samples will be collected on days 4, 7 (+1 day), 11 (+2 days), 17 (+2 days), and 21 (+2 days). The at-home stool samples will be collected by the subject into a commercially purchased container containing 95% ethanol for use as a preservative.

On days 1, 3, 5, 9 (+1 day), 14 (+2 days), 28 (+2 days) and 35 (+2 days) subjects will deposit their stool in a provided collection kit and bring the entire sample to the clinic for processing by the site. Alternatively, on these days the subjects may opt to provide the specimen during the course of visit to the study center. Collection and stool processing procedures are described in the accompanying Laboratory Manual.

A post study sample will be collected on or after Day 90 for subjects as specified by the sponsor. These samples will be collected on site; collection and stool processing procedures are described in the accompanying Laboratory Manual.

Analysis of Stool Samples

Stool samples for microbiome analysis will be prepared for DNA extraction. After DNA extraction, a sample will be analyzed using species- and strain-specific quantitative PCR analysis to evaluate *B. infantis* colonization and to monitor overall shifts in the microbiome using primers

that target the universal 16S rRNA gene shared by bacteria. Selected samples may also be analyzed by next generation DNA sequencing to determine the taxonomic composition, alpha- and beta-diversity and change of each subject's microbiome during the study. Methods may include 16S and/or whole metagenomic shotgun sequencing and culture-specific methods as deemed appropriate by the Sponsor. In the event of a supposed sample mix-up, the sponsor may analyze subjects' DNA isolated from a stool sample to confirm that all stool samples provided from a subject have the same DNA footprint.

Aliquots of stool from Days 1, 3, 4, 5, 7, 9, 11, 14, 17, 21, 28, 35,will be provided to contract lab(s) with metabolomic capabilities to measure production of short chain fatty acids, levels of HMO, and additional microbial metabolites as deemed appropriate by the Sponsor.

Blood Samples

Blood samples collected at screening (≤ 90 days prior D1) will be used to determine subject eligibility and baseline results. Blood samples will be collected and tested for the purpose of safety monitoring on days 1, 3, 5, 9 (+1 day), 14 (+2 days), 28 (+2 days) and 35 (+2 days). Specifically, the following tests will be performed: CBC with differential and platelets; alkaline phosphatase, ALT, AST, LDH, total and conjugated bilirubin, albumin, and total protein for liver function; electrolytes Na, K, Cl, HCO_3, and glucose; total calcium, magnesium, and phosphate; creatinine and BUN for renal function. Blood will also be taken at screening to test for markers of immunological activity, including but not limited to, TGFβ. Immunology samples taken at screening will not be used for subject eligibility. Additional blood samples will be drawn on days 1, 3, 5, 9 (+1 day), 14 (+2 days), 28 (+2 days) and 35 (+2 days). These additional blood draws will be used to assess levels of immunological markers, levels of metabolites related to microbiome function in the serum, and levels of HMO. All blood testing for safety and immunological testing will be performed at a central clinical laboratory.

Urine Samples

Urine samples collected at screening (≤ 90 days prior to D1) and on day 1 will be used to screen for drugs of abuse. Females of child-bearing potential will also provide urine for screening pregnancy tests at screening (≤ 90 days prior D1) and on day 1. If child-bearing status is unknown, a urine pregnancy test should be performed at both visits. Urine samples will be collected on days 1, 3, 5, and 35 (+2 days) for indoxyl sulfate tests. Urine creatinine tests will

also be conducted on days 1, 3, 5, and 35 (+2 days), the results will be used in conjunction with the indoxyl sulfate tests. Urine samples will be aliquoted and frozen at -70 deg C or colder immediately after collection. All urine samples for indoxyl sulfate testing will be sent to at a central clinical laboratory for testing.

Vital Signs and Physical Exams

Vitals signs, including temperature, blood pressure (BP), pulse, and respiration rate (RR), will be taken at screening (≤ 90 days prior to D1), day 1 and 35 (+2 days) . Subjects should rest for at least 10 mins prior to taking vitals. Vitals should be taken prior to any blood draws and in a sitting or supine position. Height, weight, and body mass index (BMI) will be taken only at the screening visit (≤ 90 days prior to D1). Physical exams will be performed at screening (≤ 90 days prior to D1) and at the day 35 visit.

Cheek Swabs

A cheek swab will be taken on day 1 of the study to determine secretor status of an individual by testing of a single region of DNA. Secretor status may impact the subject's response to treatment which is the reason for this test.

Subject Stool compliance

Subjects will be considered evaluable if each subject provides 80% of stool samples through day 35, including at least 1 stool sample from day 1, and at least 1 sample from days 3 through 5. Subjects who do not meet this criterion will be replaced.

Subject IP compliance

Subject compliance will be evaluated as follows:

- Subjects will return all IP and medication (used and unused containers).
- Subjects will record the date and times that the subject ingested IP and medication in a take home journal.
- The study site will use a defined mechanism (daily phone call and/or text messaging and/ daily email) to remind subject regarding daily dose and to gain confirmation that dosing has been completed. Any phone, text and/or email interactions will be documented as part of the study records.
- In addition, the metabolomic measurement of HMO and the quantitative measurement of *B. infantis* will provide additional evidence of subject compliance (albeit after the fact).

Subject non-compliance will be defined as a *B. infantis* signal below the limit of detection (LOD) of the qPCR assay on days 3 – 14 during the dosing period. We are setting the LOD at 27 copies per nanogram DNA. The LOD was determined using the FDA validation requirements, which defines LOD as the lowest value that produces positive results amongst 12 replicates of the same sample using dilution series of genomic DNA isolated from *B. infantis*. The LOD was increased 35% to account for variability across operators, which is in compliance with FDA repeatability and reproducibility standards. If a subject's sample yields a value lower than determined LOD, including no amplification, there is no *B. infantis* detected and the subject is deemed non-compliant. These criteria hold for every sample collected during the dosing period of *B. infantis* (days 3 – 14).

- Subjects who miss a dose of *B. infantis,* HMO or both will make up the missed dosed by adding one additional *B. infantis,* HMO, or both to their dosing regimen. No more than 2 consecutive missed doses or 3 total missed are allowed over the study. Subjects who do not meet these criteria may be replaced.
- Subjects who miss any doses of either antibiotic (metronidazole and/or vancomycin) in the first three days will be removed from the study. No more than 2 doses may be missed after the ninth dose. Subjects who do not meet these criteria may be replaced.

Safety Stopping Rules

- As the use of antibiotics in this protocol is intended to disrupt the intestinal microbiome of the subjects, it is anticipated that changes in bowel habits and/or other abdominal symptoms may ensue. A subject presenting with bloody diarrhea or severe abdominal pain with diarrhea shall be removed from the study and replaced. The study will be paused if 2 or more subjects are removed due to bloody diarrhea or severe abdominal pain with diarrhea. In collaboration with the study investigators, the Sponsor will determine if the antibiotic regimen will be changed to address this issue, or if the study will be terminated.
- In the presence of diarrhea (whereby diarrhea is defined as two or more episodes of watery and/ or unformed stool within 24 hours) without blood, and with or without mild to moderate abdominal pain, antibiotic dosing will be discontinued, but the subject will remain on study.

Study endpoints

A data collection spreadsheet will be provided in order to capture the relevant information that will be obtained by the clinical site as indicated below. For primary endpoint and microbiome /metabolomic data, the Sponsor will be responsible for capturing data and incorporating into an appropriate database.

a) Primary Endpoint
   - The primary endpoint will be changes in the level of *B. infantis* from baseline evaluated by quantitative PCR using strain specific primers. Quantitation limits will be demonstrated by qualification assays demonstrating the lower limit of detection in order to establish minimum log-fold change in *B. infantis*, as it is expected that adult subjects will not harbor *B. infantis* in baseline samples.

b) Secondary Endpoints
   - Changes in stool microbiota will be measured as well as dynamic changes in the gut community structure. These changes will be evaluated by next generation sequencing using proportions of key bacterial operational taxonomic units (OTUs), relative abundance of various taxa, diversity (alpha and beta) and stability of communities and functional metabolomic changes. Genera- and family-specific primers will also be used to perform qPCR to gauge changes in the microbial community structure.
   - Changes in viability of Proteobacteria and *Enterococcus* will be determined by plating on selective media. These measurements will be predictive for success in suppressing pathogenic bacterial relatives in future trials.
   - Changes in the carriage load of antibiotic resistance genes will also be monitored using a set of qPCR primers and probes targeting antibiotic resistance genes.

c) Blood parameters, including cytokine levels, will be evaluated at the screening visit and on study days 1, 3, 5, 9, 14, 28 and 35. Serum will also be assessed for metabolite levels, including but not limited to HMO, short chain fatty acids, and indole.

d) Secretor status will be determined by analyzing DNA collected via a cheek swab

e) Adverse events will be summarized by severity and relationship to study product. All adverse events will be reported on the study case report form designated for this purpose. Any serious and unexpected adverse event determined by the investigator to be definitely or highly likely

causally related with the consumption of IP should be reported immediately (but no later than within 24 hours) to the Sponsor's Medical Monitor.

Statistical evaluation

Since this is an initial study of the use of antibiotics and HMO in combination with *B. infantis*, it is, by definition, hypothesis-generating. Therefore, all statistical analyses will be descriptive. The various indices reflecting the microbiome will be summarized with appropriate descriptive statistics at each day of collection and compared qualitatively across time and by dosing group. Other quantitative outcome data collected will be summarized by means/medians and standard deviations/inter-quartile ranges. Graphics such as box plots also will be presented. Safety data (tolerability and adverse events) will be presented in tables with proportions (per subject) by dose and overall. A more detailed Statistical Analysis Plan will be written and finalized before the completion of this study.

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APPENDICES

APPENDIX 1 SCHEDULE OF EVENTS

All activities for Dosing Groups 1 through 5 and the Post Study sample collection (for sponsor identified subjects only) are outlined in the Schedule of Events tables as follows.

DOSING GROUP 1 SCHEDULE OF EVENTS Days 1 – 14

Dosing Group 1 Events	Screening	Dosing Days					Washout Period								
	D0 (≤ 90 days prior D1	D1	D2	D3	D4	D5	D6	D7 (+1day)	D8	D9 (+1day)	D10	D11 (+2days)	D12	D13	D14 (+2days)
Clinic visit	X	X		X		X				X					X
Final Visit															
Informed consent	X														
Review IE Criteria	X	X													
Medical History	X														
Physical Exam	X														
Demographics (age, gender, ethnicity and race	X														
Vital Signs (Temp, BP, pulse, RR)	X	X													
Height, weight and BMI	X														
Prior & Con Meds	X	X													
AEs		X		X		X				X					X
Randomization		X													
vancomycin 250 mg + metronidazole 500 mg (Q8 hours =/- 3 hours)		X	X	X	X	X									
Assess IP and Stool compliance		X	X	X	X	X									
Patient Journal (Daily Dosing, Bristol Stool Score, and Flavor Questionnaire)		X	X	X	X	X	X	X	X	X	X	X	X	X	X
Patient Phone Calls		X	X	X	X	X	X	X	X	X	X	X	X	X	X
Urine Drug Screen Test	X	X													
Urine Pregnancy Test (Females of child-bearing potential)	X	X													
Urine Creatinine Test		X		X		X									
Frozen Urine aliquots (central lab - Indoxyl Sulfate)		X		X		X									
Hematology & Chemistry testing	X	X		X		X				X					X
Serum aliquots	X	X		X		X				X					X
C-diff and VRE Stool Screening Test	X														
Stool aliquots (no preservatives)	X	X		X		X				X					X
Stool aliquots (with ethanol)					X			X				X			
Cheek Swab for Secretor Status		X													

DOSING GROUP 1 SCHEDULE OF EVENTS Days 15 – 35

Dosing Group 1 Events	Washout Period														
	D15	D16	D17 (+2days)	D18	D19	D20	D21 (+2days)	D22	D23	D24	D25	D26	D27	D28 (+2days)	D35 (+2days)
Clinic visit														X	X
Final Visit															X
Informed consent															
Review IE Criteria															
Medical History															
Physical Exam															X
Demographics (age, gender, ethnicity and race															
Vital Signs (Temp, BP, pulse, RR)															X
Height, weight and BMI															
Prior & Con Meds															X
AEs														X	X
Randomization															
vancomycin 250 mg + metronidazole 500 mg (Q8 hours =/- 3 hours)															
Assess IP and Stool compliance															X
Patient Journal (Daily Dosing, Bristol Stool Score, and Flavor Questionnaire)	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Patient Phone Calls	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
Urine Drug Screen Test															
Urine Pregnancy Test (Females of child-bearing potential)															
Urine Creatinine Test															X
Frozen Urine aliquots (central lab - Indoxyl Sulfate)															X
Hematology & Chemistry testing														X	X
Serum aliquots														X	X
C-diff and VRE Stool Screening Test															
Stool aliquots (no preservatives)														X	X
Stool aliquots (with ethanol)			X				X								
Cheek Swab for Secretor Status															

DOSING GROUP 2 SCHEDULE OF EVENTS Days 1 – 14

Dosing Group 2 Events	Screening	Dosing Days													
	D0 (≤ 90 days prior D1	D1	D2	D3	D4	D5	D6	D7 (+1day)	D8	D9 (+1day)	D10	D11 (+2days)	D12	D13	D14 (+2days)
Clinic visit	X	X		X		X				X					X
Final Visit															
Informed consent	X														
Review IE Criteria	X	X													
Medical History	X														
Physical Exam	X														
Demographics (age, gender, ethnicity and race	X														
Vital Signs (Temp, BP, pulse, RR)	X	X													
Height, weight and BMI	X														
Prior & Con Meds	X	X													
AEs		X		X		X				X					X
Randomization		X													
vancomycin 250 mg + metronidazole 500 mg (Q8 hours =/- 3 hours)		X	X	X	X	X									
B. infantis (QD)		X	X	X	X	X	X	X	X	X	X	X	X	X	X
HMO PBCLN-010 (BID)		X	X	X	X	X	X	X	X	X	X	X	X	X	X
Assess IP and Stool compliance		X	X	X	X	X									X
Patient Journal (Daily Dosing, Bristol Stool Score, and Flavor Questionnaire)		X	X	X	X	X	X	X	X	X	X	X	X	X	X
Patient Phone Calls		X	X	X	X	X	X	X	X	X	X	X	X	X	X
Urine Drug Screen Test	X	X													
Urine Pregnancy Test (Females of child-bearing potential)	X	X													
Urine Creatinine Test		X		X		X									
Frozen Urine aliquots (central lab - Indoxyl Sulfate)		X		X		X									
Hematology & Chemistry testing	X	X		X		X				X					X
Serum aliquots	X	X		X		X				X					X
C-diff and VRE Stool Screening Test	X														
Stool aliquots (no preservative)	X	X		X		X				X					X
Stool aliquots (with ethanol)					X			X				X			
Cheek Swab for Secretor Status		X													

DOSING GROUP 2 SCHEDULE OF EVENTS Days 15 – 35

Dosing Group 2 Events	Dosing Days														Washout
	D15	D16	D17 (+2days)	D18	D19	D20	D21 (+2days)	D22	D23	D24	D25	D26	D27	D28 (+2days)	D35 (+2days)
Clinic visit														X	X
Final Visit															X
Informed consent															
Review IE Criteria															
Medical History															
Physical Exam															X
Demographics (age, gender, ethnicity and race															
Vital Signs (Temp, BP, pulse, RR)															X
Height, weight and BMI															
Prior & Con Meds															X
AEs														X	X
Randomization															
vancomycin 250 mg + metronidazole 500 mg (Q8 hours =/- 3 hours)															
B. infantis (QD)															
HMO PBCLN-010 (BID)	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
Assess IP and Stool compliance														X	X
Patient Journal (Daily Dosing, Bristol Stool Score, and Flavor Questionnaire)	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
Patient Phone Calls	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
Urine Drug Screen Test															
Urine Pregnancy Test (Females of child-bearing potential)															
Urine Creatinine Test															X
Frozen Urine aliquots (central lab - Indoxyl Sulfate)															X
Hematology & Chemistry testing														X	X
Serum aliquots														X	X
C-diff and VRE Stool Screening Test															
Stool aliquots (no preservative)														X	X
Stool aliquots (with ethanol)			X				X								
Cheek Swab for Secretor Status															

DOSING GROUP 3 SCHEDULE OF EVENTS Days 1-14

Dosing Group 3 Events	Screening	Dosing Days													
	D0 (≤ 90 days prior D1	D1	D2	D3	D4	D5	D6	D7 (+1day)	D8	D9 (+1day)	D10	D11 (+2days)	D12	D13	D14 (+2days)
Clinic visit	X	X		X		X				X					X
Final Visit															
Informed consent	X														
Review IE Criteria	X	X													
Medical History	X														
Physical Exam	X														
Demographics (age, gender, ethnicity and race	X														
Vital Signs (Temp, BP, pulse, RR)	X	X													
Height, weight and BMI	X														
Prior & Con Meds	X	X													
AEs		X		X		X				X					X
Randomization		X													
vancomycin 250 mg + metronidazole 500 mg (Q8 hours =/- 3 hours)		X	X	X	X	X									
B. infantis (QD)		X	X	X	X	X	X	X	X	X	X	X	X	X	X
Assess IP and Stool compliance		X	X	X	X	X									X
Patient Journal (Daily Dosing, Bristol Stool Score, and Flavor Questionnaire)		X	X	X	X	X	X	X	X	X	X	X	X	X	X
Patient Phone Calls		X	X	X	X	X	X	X	X	X	X	X	X	X	X
Urine Drug Screen Test	X	X													
Urine Pregnancy Test (Females of child-bearing potential)	X	X													
Urine Creatinine Test		X		X		X									
Frozen Urine aliquots (central lab - Indoxyl Sulfate)		X		X		X									
Hematology & Chemistry testing	X	X		X		X				X					X
Serum aliquots	X	X		X		X				X					X
C-diff and VRE Stool Screening Test	X														
Stool aliquots (no preservative)	X	X		X		X				X					X
Stool aliquots (with ethanol)					X			X				X			
Cheek Swab for Secretor Status		X													

DOSING GROUP 3 SCHEDULE OF EVENTS Days 15 - 35

Dosing Group 3 Events	Washout														
	D15	D16	D17 (+2days)	D18	D19	D20	D21 (+2days)	D22	D23	D24	D25	D26	D27	D28 (+2days)	D35 (+2days)
Clinic visit														X	X
Final Visit															X
Informed consent															
Review IE Criteria															
Medical History															
Physical Exam															X
Demographics (age, gender, ethnicity and race															
Vital Signs (Temp, BP, pulse, RR)															X
Height, weight and BMI															
Prior & Con Meds															X
AEs														X	X
Randomization															
vancomycin 250 mg + metronidazole 500 mg (Q8 hours =/- 3 hours)															
B. infantis (QD)															
Assess IP and Stool compliance															X
Patient Journal (Daily Dosing, Bristol Stool Score, and Flavor Questionnaire)	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Patient Phone Calls	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
Urine Drug Screen Test															
Urine Pregnancy Test (Females of child-bearing potential)															
Urine Creatinine Test															X
Frozen Urine aliquots (central lab - Indoxyl Sulfate)															X
Hematology & Chemistry testing														X	X
Serum aliquots														X	X
C-diff and VRE Stool Screening Test															
Stool aliquots (no preservative)														X	X
Stool aliquots (with ethanol)			X				X								
Cheek Swab for Secretor Status															

DOSING GROUP 4 SCHEDULE OF EVENTS Days 1 - 14

Dosing Group 4 Events	Screening	Dosing Days													
	D0 (≤ 90 days prior D1	D1	D2	D3	D4	D5	D6	D7 (+1day)	D8	D9 (+1day)	D10	D11 (+2days)	D12	D13	D14 (+2days)
Clinic visit	X	X		X		X				X					X
Final Visit															
Informed consent	X														
Review IE Criteria	X	X													
Medical History	X														
Physical Exam	X														
Demographics (age, gender, ethnicity and race	X														
Vital Signs (Temp, BP, pulse, RR)	X	X													
Height, weight and BMI	X														
Prior & Con Meds	X	X													
AEs		X		X		X				X					X
Randomization		X													
vancomycin 250 mg + metronidazole 500 mg (Q8 hours =/- 3 hours)		X	X	X	X	X									
B. infantis (QD)		X	X	X	X	X	X	X	X	X	X	X	X	X	X
HMO PBCLN-010 (BID)		X	X	X	X	X	X	X	X	X	X	X	X	X	X
HMO 2'FL + LNnT (Q12 hours)															
Assess IP and Stool compliance		X	X	X	X	X									X
Patient Journal (Daily Dosing, Bristol Stool Score, and Flavor Questionnaire)		X	X	X	X	X	X	X	X	X	X	X	X	X	X
Patient Phone Calls		X	X	X	X	X	X	X	X	X	X	X	X	X	X
Urine Drug Screen Test	X	X													
Urine Pregnancy Test (Females of child-bearing potential)	X	X													
Urine Creatinine Test		X		X		X									
Frozen Urine aliquots (central lab - Indoxyl Sulfate)		X		X		X									
Hematology & Chemistry testing	X	X		X		X				X					X
Serum aliquots	X	X		X		X				X					X
Stool aliquots (no preservative)	X	X		X		X				X					X
Stool aliquots (with ethanol)					X			X				X			
Cheek Swab for Secretor Status		X													

DOSING GROUP 4 SCHEDULE OF EVENTS Days 15 - 35

Dosing Group 4 Events	Dosing Days														Washout
	D15	D16	D17 (+2days)	D18	D19	D20	D21 (+2days)	D22	D23	D24	D25	D26	D27	D28 (+2days)	D35 (+2days)
Clinic visit														X	X
Final Visit															X
Informed consent															
Review IE Criteria															
Medical History															
Physical Exam															X
Demographics (age, gender, ethnicity and race															
Vital Signs (Temp, BP, pulse, RR)															X
Height, weight and BMI															
Prior & Con Meds															X
AEs														X	X
Randomization															
vancomycin 250 mg + metronidazole 500 mg (Q8 hours =/- 3 hours)															
B. infantis (QD)															
HMO PBCLN-010 (BID)															
HMO 2'FL + LNnT (Q12 hours)	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
Assess IP and Stool compliance														X	X
Patient Journal (Daily Dosing, Bristol Stool Score, and Flavor Questionnaire)	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Patient Phone Calls	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
Urine Drug Screen Test															
Urine Pregnancy Test (Females of child-bearing potential)															
Urine Creatinine Test															X
Frozen Urine aliquots (central lab - Indoxyl Sulfate)															X
Hematology & Chemistry testing														X	X
Serum aliquots														X	X
Stool aliquots (no preservative)														X	X
Stool aliquots (with ethanol)			X				X								
Cheek Swab for Secretor Status															

DOSING GROUP 5 SCHEDULE OF EVENTS Days 1 -14

Dosing Group 5 Events	Screening	Dosing Days													
	D0 (≤ 90 days prior D1)	D1	D2	D3	D4	D5	D6	D7 (+1day)	D8	D9 (+1day)	D10	D11 (+2days)	D12	D13	D14 (+2days)
Clinic visit	X	X		X		X				X					X
Final Visit															
Informed consent	X														
Review IE Criteria	X	X													
Medical History	X														
Physical Exam	X														
Demographics (age, gender, ethnicity and race	X														
Vital Signs (Temp, BP, pulse, RR)	X	X													
Height, weight and BMI	X														
Prior & Con Meds	X	X													
AEs		X		X		X				X					X
Randomization		X													
vancomycin 250 mg + metronidazole 500 mg (Q8 hours =/- 3 hours)		X	X	X	X	X									
B. infantis (QD)		X	X	X	X	X	X	X	X	X	X	X	X	X	X
HMO 2'FL + LNnT (Q12 hours)		X	X	X	X	X	X	X	X	X	X	X	X	X	X
Assess IP and Stool compliance		X	X	X	X	X									X
Patient Journal (Daily Dosing, Bristol Stool Score, and Flavor Questionnaire)		X	X	X	X	X	X	X	X	X	X	X	X	X	X
Patient Phone Calls		X	X	X	X	X	X	X	X	X	X	X	X	X	X
Urine Drug Screen Test	X	X													
Urine Pregnancy Test (Females of child-bearing potential)	X	X													
Urine Creatinine Test		X		X		X									
Hematology & Chemistry testing	X	X		X		X				X					X
Serum aliquots	X	X		X		X				X					X
C-diff and VRE Stool Screening Test	X														
Stool aliquots (no preservative)	X	X		X		X				X					X
Stool aliquots (with ethanol)					X			X				X			
Cheek Swab for Secretor Status		X													

DOSING GROUP 5 SCHEDULE OF EVENTS Days 15 – 35

Dosing Group 5 Events	Dosing Days														Washout
	D15	D16	D17 (+2days)	D18	D19	D20	D21 (+2days)	D22	D23	D24	D25	D26	D27	D28 (+2days)	D35 (+2days)
Clinic visit														X	X
Final Visit															X
Informed consent															
Review IE Criteria															
Medical History															
Physical Exam															X
Demographics (age, gender, ethnicity and race															
Vital Signs (Temp, BP, pulse, RR)															X
Height, weight and BMI															
Prior & Con Meds															X
AEs														X	X
Randomization															
vancomycin 250 mg + metronidazole 500 mg (Q8 hours =/- 3 hours)															
B. infantis (QD)															
HMO 2'FL + LNnT (Q12 hours)	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
Assess IP and Stool compliance														X	X
Patient Journal (Daily Dosing, Bristol Stool Score, and Flavor Questionnaire)	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Patient Phone Calls	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
Urine Drug Screen Test															
Urine Pregnancy Test (Females of child-bearing potential)															
Urine Creatinine Test															X
Hematology & Chemistry testing														X	X
Serum aliquots														X	X
C-diff and VRE Stool Screening Test															
Stool aliquots (no preservative)														X	X
Stool aliquots (with ethanol)			X				X								
Cheek Swab for Secretor Status															

POST STUDY SAMPLE SCHEDULE OF EVENTS (SPONSOR SELECTED SUBJECTS ONLY)

Post Study Sample Events	Post Study Sample - Sponsor Selected Subjects only
	D90 or later
Clinic visit	X
Final Visit	X
Informed consent	X
Prior Meds - Antibiotics (only) taken since D35	X
Stool aliquots (no preservative)	X