Official Title:	Dietary Study of a Complex Oligosaccharide With and Without a Probiotic in Healthy Volunteers
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TITLE PAGE

CLINICAL STUDY PROTOCOL

PROTOCOL TITLE:	**Dietary Study of Human Milk Oligosaccharides Concentrate PBCLN-005 with and without *Bifidobacterium infantis* in Healthy Adult Volunteers**
PROTOCOL NUMBER:	**20-CT-001 – 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**

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David J Rechtman, M.D.

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.

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INVESTIGATOR PROTOCOL AGREEMENT

The signature below constitutes that I agree to the following:

- I have reviewed the protocol and the attachments.
- This trial 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 RATIONALE

Dietary Study of Human Milk Oligosaccharide Concentrate PBCLN-005 with and without *B. infantis* in Healthy Adult Volunteers

Background

Human milk oligosaccharide (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.[i,ii,iii] Approximately 200 different HMO structures have been identified, although any particular mother will only produce a subset of those. Studies have shown that HMOs can alter the gut microbiome by favoring the growth of certain organisms as well as, perhaps, by inhibiting others.[4] It has also been demonstrated that HMOs can act as "decoy" receptors for a variety of pathogens and toxins.[iv] They have also been shown, in vitro, to directly interact with certain components of the adaptive immune system.[4]

As a result of the aforementioned activities it is 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 HMOs 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 might include conditions as disparate as inflammatory bowel disease and stem cell transplantation.

We hypothesize that *B. longum* subspecies *infantis* (*B. infantis*) might be a particularly beneficial organism to expand in the microbiome when managing such diseases in combination with HMOs. 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.[7] These dietary effects are postulated to be due to metabolism of HMOs by *B. infantis*, as infants receiving human milk in the absence of *B. infantis* don't show these effects. Importantly, *B. infantis* is not observed as a constituent of the human adult gut microbiota so it may therefore be necessary to administer both HMOs and *B. infantis* to obtain the full beneficial impact of HMOs feeding in adults.

Prolacta Bioscience manufactures human milk-based products from donated breast milk. In the course of the manufacture of human milk-based human milk fortifier, skim breast milk is ultra-filtered in order to concentrate proteins. Investigation of the permeate produced through this process demonstrated that human milk oligosaccharides were present in amounts comparable to those found in raw breast milk. Moreover, as the starting material consists of breast milk pooled from between 100 and 200 mothers, the full range of HMOs could be found. Subsequently, a method was developed to allow for the production of material with elevated concentrations of HMOs which contains little to no residual lactose through the introduction of lactase which is then removed.

An unconcentrated, pasteurized form of HMO material containing a proportional amount of lactose has already been used in an NIH sponsored clinical trial in premature infants with no reported ill effects.[v] Prolacta Bioscience has also completed a healthy volunteer study using the same HMO material as in this trial (with no addition of *B. infantis*) which showed good tolerability. No treatment emergent adverse effects were reported over the course of the trial.[vi]

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).[8]

Hypothesis

The primary goal of this study is to evaluate the synergy of PBCLN-005 in varying doses with a fixed dose of *B. infantis*, compared with either component of the IP given alone. Synergy is defined as the enhanced gastrointestinal colonization of *B. infantis* upon feeding with HMO feeding. Secondary endpoints will measure changes in the gut microbiome, fecal metabolome and the subject's immune status as measured by systemic markers. We hypothesize that the combination will result in greater proliferation of *B. infantis* than was seen with the HMO concentrate alone.

Another objective is to understand the role of the low pH of the stomach in colonization of B. infantis. For most non-spore forming gut bacteria, the stomach is a barrier to colonization since acid exposure can reduce bacterial titer by 1,000- to 1,000,000-fold. While some subjects in the study will take a proton pump inhibitor (PPI; Zegerid), or a proton pump inhibitor (Zegerid) and

histamine H2-receptor antagonist (H2 blocker: Pepcid) to transiently reduce the production of gastric acid prior to *B. infantis* ingestion, some study groups will ingest *B. infantis* without PPI pretreatment. It was initially hypothesized that PPI might be necessary to observe *B. infantis* colonization distal to the acid milieu of the stomach. Preliminary data from the first group of subjects tested suggest that omeprazole may be inhibitory to *B. infantis* colonization and that colonization occurs in the absence of gastric acid suppression. *In vitro* data suggest that the inhibitory effect is not seen with the histamine H2-receptor antagonist famotidine. Therefore, this has resulted in a change to this hypothesis. We now seek to determine if colonization occurs in the presence of an acidic milieu in the stomach, if the apparent inhibitory effect of PPI on colonization is real and if the prediction that the effect will not be seen with famotidine is correct because all target group subjects for future studies are on acid blockers.

We also hypothesize that ingestion of PBCLN-005 along with the *B. infantis* will result in a more marked change in immune markers than was seen in the previous study in healthy adults. 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.

Methods

We propose to conduct an unblinded six cohort multi-dose trial using 10 subjects per cohort for a total of 60 healthy adult volunteers ages 18-44 in order to evaluate the effects of the use of HMO Concentrate (PBCLN-005) and *B. infantis* on the human microbiome.

Six separate cohorts of both male and female study subjects will receive some form of the IP. All subjects in Cohort groups 1-4 and 6 will be followed from the day of the first dose of IP (day 1) until 28 days (day 29) after the first dose. Subjects in Cohort 1 will consume *B infantis* once per day for 7 days. Subjects in Cohort 2-4 and Cohort 6 will consume the IP twice per day for 14 consecutive days. Cohorts 1-4 and Cohort 6 will be followed until the 28th day (day 29) following the first day of the feeding protocol (day 1).

Subjects in Cohort 5 will receive two courses of IP; the first course will be accompanied with PPI. During the second course, some subjects will receive a H2 blocker, and some subjects will not. Subjects will be followed from the day of the first dose of study drug (day 1) until 28 days after the first dose (day 29), and then from the first day of their second course (day 29) until the

28th day following the first dose of that course (day 57). Subjects in that cohort will consume the HMO concentrate twice per day for 14 consecutive days during both courses of IP and will be followed until the 28th day following the first day of the second course of the feeding protocol for a total of 57 days.

IP will be packaged so that consumption will be one container of each component per dose, to be mixed immediately prior to ingestion, except for Cohorts 1 and 2 where the IP will consist of only one container per dose.

The PBCLN-005 will be presented in frozen liquid form while the *B. infantis* will be presented in a foil package as a lyophilized powder to be mixed with thawed PBCLN-005 or an appropriate alternative diluent prior to ingestion.

Each bottle of HMO concentrate will be filled to deliver the required amount of HMO. The daily target doses of concentrated oligosaccharide to be evaluated in this study are 4.5g, 9g and 18g. The daily dose of *B. infantis* is $\geq 8X10^7$ CFU in a foil pouch. The dosing cohort regime is outlined as follows.

1. Study subjects randomized to Cohort 1 will consume by mouth *B. infantis* once per day for 7 consecutive days. Subjects will mix *B. infantis* in an aqueous diluent supplied by the pharmacy
2. Study subjects randomized to Cohort 2 will consume by mouth the HMO investigational product (IP) twice per day for 14 consecutive days.
3. Study subjects randomized to Cohort 3 will consume by mouth HMO investigational product (IP) twice per day for 14 consecutive days. *B. infantis* will be taken once per day for 7 consecutive days. The subject will mix HMO and *B. infantis* On days 8-14, only HMO product will be consumed (*B. infantis* will not be taken).
4. Study subjects randomized to Cohort 4 will consume by mouth HMO investigational product (IP) twice per day for 14 consecutive days. *B. infantis* will be taken once per day for 7 consecutive days. The subject will mix HMO and *B. infantis*. On days 8-14, only HMO product will be consumed (*B. infantis* will not be taken).
5. Study subjects randomized to Cohort 5 will undergo two consecutive dosing courses. During the first dosing course, they will consume by mouth HMO investigational product

(IP) twice per day for 14 consecutive days. PPI and *B. infantis* will be taken once per day for 7 consecutive days. On dosing days 1-7 the first dose of HMO will be preceded by one hour and up to two hours by a single dose of PPI to protect the *B. infantis* from the acid milieu of the stomach. The subject will mix HMO and *B. infantis* and ingest at least one hour after taking the PPI. On days 8-14, only HMO product will be consumed (neither the PPI nor *B. infantis* will be taken). Cohort 5 will undergo a second dosing course from days 29-42. For all subjects, the HMO and *B. infantis* will be taken in the same manner as the first dosing course. Note: Prior to Protocol Revision 4, two subjects have already been dosed in Cohort 5. The first four of the remaining subjects yet to be enrolled in this cohort will take an H2 blocker in the same manner as the PPI in the first dosing course. The remaining four subjects enrolled into Cohort 5 will not take any PPI or H2 blocker during the second dosing course.

6. Study subjects randomized to Cohort 6 will consume by mouth HMO investigational product (IP) twice per day for 14 consecutive days and *B. infantis* will be taken once per day for 7 consecutive days. On dosing days 1-7 the subject will mix one dose of HMO and *B. infantis* ingest. On days 8-14, only HMO product will be consumed (the *B. infantis* will not be taken).

Table 1 Dosing cohorts

Cohort	Target HMO Amount (days 1-14)	PPI **Cohort 5 Only** (Course 1 = Days 1-7)	H2 Blocker **Cohort 5 Only** (Course 2 = Days 29 – 35)	*B. infantis* (days 1-7)
1	None	No	N/A	Yes, one sachet
2	18g	No	N/A	None
3	4.5g	No	N/A	Yes, one sachet
4	9g	No	N/A	Yes, one sachet
5[A]	18g	Yes	Yes (4 subjects) No (4 subjects)	Yes, one sachet
6	18g	No	N/A	Yes, one sachet

[A] This group will undergo two dosing courses. One from day 1-14 and one from day 29-42. Four of the subjects in the second course will be assigned a H2 blocker and four of the subjects will not take any PPI or H2 blocker.
NOTE: Prior to Protocol Revision 4, two subjects were enrolled in Cohort 5 based on the original dosing scheme. Thus, two subjects have already been dosed in Cohort 5. The first four of the remaining subjects yet to be dosed in the Cohort 5 second dosing course will take a H2 blocker in the same manner as the PPI in the first dosing course. The remaining four subjects enrolled into Cohort 5 will not take any PPI or H2 blocker during the second dosing course.

As the maximum quantity of human milk oligosaccharides in this study has previously been given to healthy adult volunteers with good tolerance and no treatment emergent adverse events[6], this study will not be conducted in the manner of a dose escalation safety study. That is, the randomization scheme will allow for the assignment of any given study subject to any of the six cohorts rather than to fill the cohorts sequentially.

Samples of blood, urine and stool from all subjects will be taken at screening (day 0) to determine subject eligibility and to establish baseline values. Urine will only be required at screening. Stool samples will also be required on days 1, 5, 8, 15, 22 and 29, where day 1 is the first day of the feeding protocol. Blood samples will also be drawn on days 1, 8, 15, and 29. For dose group 5 ONLY, stool samples will also be required on days 33, 36, 43, 50 and 57 and blood samples on days 36, 43 and 57. Details regarding type of stool sample and its processing is described in the accompanying Laboratory Manual.

The summary of all study events is presented in Table 2 at the end of this protocol.

Sample Size

Six groups of 10 subjects will be included in this study for a total sample size of 60. The sample size was not determined statistically, but rather represents a typical number for an exploratory study of healthy volunteers.

Study Duration

Six separate cohorts of both male and female study subjects will receive some form of the IP. All subjects in Cohorts 1-4 and 6 will be followed from the day of the first dose of IP (day 1) until 28 days (day 29) after the first dose. Subjects in Cohort 1 will consume *B infantis* once per day for 7 days. Subjects in Cohort 2-4 and Cohort 6 will consume the IP twice per day for 14 consecutive days. Cohorts 1-4 and Cohort 6 will be followed until the 28th day (day 29) following the first day of the feeding protocol (day 1).

Subjects in Cohort 5 will receive two courses of IP. They will be followed from the day of the first dose of study drug (day 1) until 28 days after the first dose (day 29), and then from the first day of their second course (day 29) until the 28th day following the first dose of that course (day 57). Subjects in that cohort will consume the HMO concentrate twice per day for 14 consecutive days during both courses of IP and will be followed until the 28th day following the first day of the second course of the feeding protocol for a total of 57 days.

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 18-44 years (subjects must be 18-44 at the time of consent)
- Willingness to complete study specific questionnaires
- Willingness to complete journal to record IP dosing times
- Willingness to complete all study procedures and clinic visits, and provide required samples
- Able to provide stool samples on specific study days

- Willingness to collect and process stool samples at home and transport stool samples to clinic
- Bristol Stool Score of 1 through 5 at time of screening and on day 1, subjects must verbally confirm that they did not have stool conforming to Bristol Stool Score 6 or 7 during the 7 days prior to dosing.
- Able to store up to 9 days of IP in refrigerator
- 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
- Provides informed consent

Exclusion Criteria

- Women who are pregnant or breastfeeding, or intend to become pregnant during the course of this study
- Subjects with allergy to, or other reason for contraindication of the PPIs or H2 blocker* chosen for this study.
    - *The H2 blocker allergy or contraindication exclusion only applies to Subjects enrolled in Cohort 5 that are scheduled to receive an H2 blocker. Cohort 5 Subjects with an H2 blocker allergy or contraindication may be assigned to the second dosing course without an acid blocker.
- Subjects with history of lactose intolerance
- Subjects who are on a PPI regimen
- Subject who has taken a probiotic during the previous 30 days prior to first dose, or intends to take a probiotic during the study
- Subject who has taken antibiotics within 120 days prior to first dose

- Alcohol or drug abuse during the last 12 months, including passing a screen for drugs of abuse at screening
- Unstable medical condition, in the opinion of the investigator
- Clinically significant abnormal laboratory test results at screening
- Subjects who are unable or unwilling to provide stool samples on a regular basis
- 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 *B. infantis* prior to study start, as determined by qPCR of stool Patients with pending screening test results will be allowed to proceed.
- Bristol Stool Score of 6 or 7 at time of screening and on day 1, subject reported Bristol Stool Scores of 6 or 7 for any of the 7 days prior to dosing. If there is difficulty distinguishing between a Bristol Stool score of 5 or 6, a Bristol Stool Score of 6 will only be assigned if the subject has a GI clinical indication.

Study Procedure

Screening Visit (day 0, may occur up to and including 90 days prior to the subject's dose. After informed consent is obtained and subject eligibility is assessed, vital signs, samples of blood, stool, and urine will be obtained from subjects at screening visit (day 0). Prior to dosing on day 1, subject eligibility will be reassessed, vital signs, samples of blood, and stool will be obtained from subjects. On days 1-14, subjects in Cohorts 2-6 will consume the HMO concentrate twice daily. On days 1-7, Cohort 5 will ingest a PPI one hour prior to ingesting the HMO concentrate or an aqueous diluent (Cohort 1 only). Additionally, on days 1-7, Cohorts 1 and 3-6, subjects will ingest *B. infantis* admixed with the HMO solution or an aqueous diluent (Cohort 1 only). For Cohort 5 only, the same pattern will be repeated on study days 29-42. The first four of the subjects yet to be enrolled in this cohort will take a H2 blocker in the same manner as the PPI in the first dosing course. The remaining four subjects enrolled into Cohort 5 will not take any PPI or H2 blocker during the second dosing course.

Samples of blood, urine and stool from all subjects will be taken at screening. Stool samples will also be required on days 1, 5, 8, 15, 22 and 29. Blood samples will also be drawn on days 1, 8, 15, and 29. For dose group 5 ONLY, additional stool samples will also be required on days 33, 36, 43, 50 and 57 and blood samples on days 36, 43 and 57.

Stool Samples

Stool samples collected at screening (day 0) will be analyzed by qPCR to determine if the subject is a carrier of *B. infantis*. Screening (day 0) stool samples collected for metabolomic and metagenomic testing will not be used to determine patient eligibility.

Stool samples will be tested for microbiome, metabolomics and metagenomics. At-home stool samples will be collected on days 5, 22, 33 and 50 (+1 day). Day 33 and 50 applies to dose group 5 ONLY. The At-home stool samples will be collected by the subject into the Omnigene-Gut OMR-200 collection tube (DNA Genotek; Ottawa, Canada). On days 1, 8, 15, 29 (+2 days, excluding day 1), and days 36, 43, and 57 (+2 days) for dose group 5 only, 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.

Microbiome 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 both during and after HMO ingestion. 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.

Aliquots of stool from Days 1, 8, 15, 29 (+2 days, excluding day 1) and days 36, 43, 57 (+2 days) for dose group 5 only, will be aliquoted and frozen at -70 deg C or colder immediately

after collection. These samples will be provided to contractor(s) with metabolomic capabilities to measure production of short chain fatty acids, levels of HMO, and other microbial metabolites as deemed appropriate by the Sponsor.

Blood Samples

Blood samples collected at screening (day 0) 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, 15, 29, (+2 days, excluding day 1) and days 43, and 57 (+2 day) for Cohort 5 ONLY. 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 immunological blood samples will be drawn on day 8 (+2 days), and on day 36 (+2 days) for Cohort 5 ONLY.

All blood testing for safety and immunological testing will be performed at a central clinical laboratory.

Urine Samples

Urine samples collected at screening (day 0) will be used to screen for drugs of abuse. Females of child-bearing potential will also provide urine for screening pregnancy tests at screening (day 0). If child-bearing status is unknown, a urine pregnancy test should be performed at screening.

Vital Signs

Vitals signs, including temperature, blood pressure (BP), pulse, and respiration rate (RR), will be taken at screening (day 0), day 1 and all follow up clinic visits days 8, 15, 29, 36, 43 and 57. Days 36, 43 and 57, only apply to subjects in Cohort 5. Subjects should rest for at least 10 mins prior to taking vitals. Vitals should be taking 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 (day 0).

Subject Stool compliance

Subjects will be considered evaluable if each subject provides 80% of samples through day 21. Subjects who do not meet this criterion will be replaced. Subjects that are identified as *B. infantis* carriers at the time of screening may be replaced depending when stool test is resulted.

Subject IP compliance

Subject compliance will be evaluated in 2-ways:

- Subjects will return all IP (used and unused containers for PBLCN-005 and *B. infantis*) to the clinical site on days 8 and 15 for reconciliation by the Pharmacy.
- Subjects will record the date and times that the subject ingested IP 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).
- Subjects who miss a dose of their PPI or H2 blocker (Cohort 5 only) or *B. infantis* or both (Cohort 5 only), will make up the missed dosed by adding one additional dosing day of PPI, H2 blocker, or *B infantis* or both to their dosing regimen. No more than 2 consecutive missed doses or 3 missed are allowed over the study. Subjects who do not meet these criteria may be replaced.
- Subjects who miss a dose of PBCLN-005, will make up the missed dosed by adding one additional dose of PBCLN-005 to their dosing regimen. No more than 2 consecutive missed doses of PBCLN-005, or 3 missed doses of PBCLN-005 are allowed total over study. For Cohort 5, this applies to each dosing period. Subjects who do not meet this criterion may be replaced.

Study endpoints

A data collection spreadsheet will be provided in order to capture the relevant information that will be obtained by the clinical site 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 primers for species and strain. 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 taxonomy units (OTUs), relative abundance of various taxa, diversity (alpha and beta) and stability of communities and functional metabolomic changes.
   - Changes in viability of proteobacteria and *Enterococcus* will be determined by plating on selective media. These measurements will predictive for success in suppressing pathogenic bacterial relatives in future trials.
   - Blood parameters, such as cytokine levels at study days -1, 8, 15 and 2 except for Cohort 5 who will also be evaluated on days 36, 43 and 57.
   - Determination of the need for protection of *B. infantis* from stomach acid in order for engraftment of the organism into the gut microbiome to occur.

c) 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.

d) Baseline measures as of study entry

e) IP Feeding Protocol

f) The dose level of PBCLN-005 consumed will be recorded as well as consumption of *B infantis*.

g) Demographics; age, gender, ethnicity and race

h) Stool changes in subject's stool per Bristol Stool Score

i) IP taste/ flavor on dosing days using a visual acuity scale

Statistical evaluation

Since this is an initial study of the use of PBCLN-005 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 dose. 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.

Schedule of Events Tables

Schedule of Events Table
Cohort 1

Protocol Number 20-CT-001																		
Schedule of Events For Cohort 1 (Day 0 through Day 29)																		
Events	**Cohort 1 Screening**	**Cohort 1 Dosing Days**															**Cohort 1 Washout**	
	D0 (≤ 90 days prior to D1)	D1	D2	D3	D4	D5 (+1D)	D6	D7	D8 (+2D)	D9	D10	D11	D12	D13	D14	D15 (+2D)	D22 (+2D)	D29 (+2D)
Clinic visit	X	X							X							X		X
Final Visit																		X
Informed consent	X																	
Review IE Criteria	X	X																
Medical History	X																	
Physical Exam	X																	X
Demographics (age, gender, ethnicity and race	X																	
Vital Signs (Temp, BP, pulse, RR)	X	X							X							X		X
Height, weight and BMI	X																	
Prior & Con Meds	X	X							X							X		X
AEs		X							X							X		X

Cohort 1 (Cont.)

Protocol Number 20-CT-001																		
Schedule of Events For Cohort 1 (Day 0 through Day 29)																		
Events	**Cohort 1 Screening**	**Cohort 1 Dosing Days**															**Cohort 1 Washout**	
	D0 (≤ 90 days prior to D1)	D1	D2	D3	D4	D5 (+1D)	D6	D7	D8 (+2D)	D9	D10	D11	D12	D13	D14	D15 (+2D)	D22 (+2D)	D29 (+2D)
Randomization		X																
Dispense IP: B. infantis (≥8x107 cfu/d) and aqueous dilute		X																
Dosing: *B. infantis* (≥8x10^7 cfu/d)		X	X	X	X	X	X	X										
Assess IP and Stool compliance									X									X
Stool Questionnaires		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Daily Dosing and Flavor Questionnaire		X	X	X	X	X	X	X										
Patient Phone Calls			X	X	X	X	X	X										

Cohort 1 (cont.)

Protocol Number 20-CT-001																		
Schedule of Events For Cohort 1 (Day 0 through Day 29)																		
Events	**Cohort 1 Screening**	**Cohort 1 Dosing Days**															**Cohort 1 Washout**	
	D0 (≤ 90 days prior to D1)	D1	D2	D3	D4	D5 (+1D)	D6	D7	D8 (+2D)	D9	D10	D11	D12	D13	D14	D15 (+2D)	D22 (+2D)	D29 (+2D)
Urine Drug Screen Test	X																	
Urine Pregnancy Test (Females of child-bearing potential)	X																	
Hematology & Chemistry testing	X	X														X		X
Immunology testing (Screening samples will not be evaluated for eligibility)	X	X							X							X		X
Stool (metagenomics) (Screening samples will not be evaluated for eligibility)	X	X				X			X							X	X	X
Stool (metabolomics) (Screening samples will not be evaluated for eligibility)	X	X							X							X		X
Stool (microbiology)	X	X							X							X		X
Fecal pH (using nitrazine paper)	X	X							X							X		X

Schedule of Events Table
Cohorts 2, 3, 4, and 6

Protocol Number 20-CT-001																		
Schedule of Events For Cohorts 2, 3, 4, & 6 (Day 0 through Day 29)																		
Events	**Cohorts 2, 3, 4, & 6 Screening**	**Cohorts 2, 3, 4, & 6 Dosing Days**															**Cohorts 2, 3, 4, & 6 Washout**	
	D0 (≤ 90 days prior to D1)	D1	D2	D3	D4	D5 (+1D)	D6	D7	D8 (+2D)	D9	D10	D11	D12	D13	D14	D15 (+2D)	D22 (+2D)	D29 (+2D)
Clinic visit	X	X							X							X		X
Final Visit																		X
Informed consent	X																	
Review IE Criteria	X	X																
Medical History	X																	
Physical Exam	X																	X
Demographics (age, gender, ethnicity and race	X																	
Vital Signs (Temp, BP, pulse, RR)	X	X							X							X		X
Height, weight and BMI	X																	
Prior & Con Meds	X	X							X							X		X
AEs		X							X							X		X

Cohorts 2, 3, 4, and 6 (cont.)

Protocol Number 20-CT-001																		
Schedule of Events For Cohorts 2, 3, 4 & 6 (Day 0 through Day 29)																		
Events	**Cohorts 2, 3, 4, & 6 Screening**	**Cohorts 2, 3, 4, & 6 Dosing Days**															**Cohorts 2, 3, 4, & 6 Washout**	
	D0 (≤ 90 days prior to D1)	D1	D2	D3	D4	D5 (+1D)	D6	D7	D8 (+2D)	D9	D10	D11	D12	D13	D14	D15 (+2D)	D22 (+2D)	D29 (+2D)
Randomization		X																
Dispense IP:, B. infantis (≥8x107 cfu/d) (except Cohort 2) (Note: On D1 dispense 9-day supply of PBCLN-005. On D8 dispense 5-day supply of PBCLN-005)		X							X									
Dosing: *B. infantis* (≥8x10^7 cfu/d) (All Cohorts except Cohort 2)		X	X	X	X	X	X	X										
Dosing: PBCLN-005 1 bottle po bid (Target 4.5g, 9g or 18g)		X	X	X	X	X	X	X	X	X	X	X	X	X	X			
Assess IP and Stool compliance		X							X							X		X
Stool Questionnaire		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Daily Dosing 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			

Cohorts 2, 3, 4 & 6 (cont.)

Protocol Number 20-CT-001																		
Schedule of Events For Cohorts 2, 3, 4, & 6 (Day 0 through Day 29)																		
Events	**Cohorts 2, 3, 4, & 6 Screening**	**Cohorts 2, 3, 4, & 6 Dosing Days**															**Cohorts 2, 3, 4, & 6 Washout**	
	D0 (≤ 90 days prior to D1)	D1	D2	D3	D4	D5 (+1D)	D6	D7	D8 (+2D)	D9	D10	D11	D12	D13	D14	D15 (+2D)	D22 (+2D)	D29 (+2D)
Urine Drug Screen Test	X																	
Urine Pregnancy Test (Females of child-bearing potential)	X																	
Hematology & Chemistry testing	X	X														X		X
Immunology testing (Screening samples will not be evaluated for eligibility)	X	X							X							X		X
Stool (metagenomics) (Screening samples will not be evaluated for eligibility)	X	X				X			X							X	X	X
Stool (metabolomics) (Screening samples will not be evaluated for eligibility)	X	X							X							X		X
Stool (microbiology)	X	X							X							X		X
Fecal pH (using nitrazine paper)	X	X							X							X		X

Schedule of Events Table
Cohort 5

Protocol Number 20-CT-001																	
Schedule of Events For Cohort 5 (Day 0 through Day 22)																	
Events	**Cohort 5 Screening**	**Cohort 5 1st Dosing Course**															**Cohort 5 1st Washout**
	D0 (≤ 90 days prior to D1)	D1	D2	D3	D4	D5 (+1D)	D6	D7	D8 (+2D)	D9	D10	D11	D12	D13	D14	D15 (+2D)	D22 (+2D)
Clinic visit	X	X							X							X	
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							X							X	
Height, weight and BMI	X																
Prior & Con Meds	X	X							X							X	
AEs		X							X							X	

Cohort 5 (Cont.)

Protocol Number 20-CT-001																	
Schedule of Events For Cohort 5 (Day 0 through Day 22)																	
Events	**Cohort 5 Screening**	**Cohort 5 1st Dosing Course**															**Cohort 5 1st Washout period**
	D0 (≤ 90 days prior to D1)	D1	D2	D3	D4	D5 (+1D)	D6	D7	D8 (+2D)	D9	D10	D11	D12	D13	D14	D15 (+2D)	D22 (+2D)
Randomization		X															
Dispense IP: PPI, B. infantis (≥8x107 cfu/d) (Note: On D1 dispense 9-day supply of PBCLN-005. On D8 dispense 5-day supply of PBCLN-005)		X							X								
Dosing: PPI		X	X	X	X	X	X	X									
Dosing: *B. infantis* (≥8x10^7 cfu/d)		X	X	X	X	X	X	X									
Dosing: PBCLN-005 1 bottle po bid (18g)		X	X	X	X	X	X	X	X	X	X	X	X	X	X		
Assess IP and Stool compliance		X							X							X	
Stool Questionnaire		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Daily Dosing 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		

Cohort 5 (Cont.)

Protocol Number 20-CT-001																	
Schedule of Events For Cohort 5 (Day 0 through Day 22)																	
Events	**Cohort 5 Screening**	**Cohort 5 1st Dosing Course**															**Cohort 5 1st Washout**
	D0 (≤ 90 days prior to D1)	D1	D2	D3	D4	D5 (+1D)	D6	D7	D8 (+2D)	D9	D10	D11	D12	D13	D14	D15 (+2D)	D22 (+2D)
Urine Drug Screen Test	X																
Urine Pregnancy Test (Females of child-bearing potential)	X																
Hematology & Chemistry testing	X	X														X	
Immunology testing (Screening samples will not be evaluated for eligibility)	X	X							X							X	
Stool (metagenomics) (Screening samples will not be evaluated for eligibility)	X	X				X			X							X	X
Stool (metabolomics) (Screening samples will not be evaluated for eligibility)	X	X							X							X	
Stool (microbiology)	X	X							X							X	
Fecal pH (using nitrazine paper)	X	X							X							X	

Cohort 5 (Cont.)

Protocol Number 20-CT-001																	
Schedule of Events For Cohorts 5 Only (Day 29 through Day 57)																	
Events	**Cohort 5 2nd Dosing Course**															**Cohort 5 2nd Washout Period**	
	D29	D30	D31	D32	D33 (+1D)	D34	D35	D36 (+2D)	D37	D38	D39	D40	D41	D42	D43 (+2D)	D50 (+2D)	D57 (+2D)
Clinic visit	X							X							X		X
Review IE Criteria[1]	X																
Final Visit																	X
Physical Exam																	X
Vital Signs (Temp, BP, pulse, RR)	X							X							X		X
Prior & Con Meds	X							X							X		X
AEs	X							X							X		X
Dispense IP: H2 blocker (if applicable)[2], B. infantis (≥8x107 cfu/d) (Note: On D29 dispense 9-day supply of PBCLN-005. On D36 dispense 5-day supply of PBCLN-005)	X[2]							X[2]									
Dosing: H2 blocker[2]	X[2]	X[2]	X[2]	X[2]	X[2]	X[2]	X[2]										
Dosing: *B. infantis* (≥8x10[7] cfu/d)	X	X	X	X	X	X	X										
Dosing: PBCLN-005 (Target 18g)	X	X	X	X	X	X	X	X	X	X	X	X	X	X			

[1]Subjects will be assessed for an H2 blocker allergy or contraindication prior to dosing in the second dosing course with the H2 blocker

[2]The first four of the subjects yet to be enrolled in this cohort will take a H2 blocker in the same manner as the PPI in the first dosing course. The remaining four subjects enrolled into Cohort 5 will not take any PPI or H2 blocker during the second dosing course.

Cohort 5 (cont.)

Protocol Number 20-CT-001																	
Schedule of Events For Cohorts 5 (Day 29 through Day 57)																	
Events	**Cohort 5 2nd Dosing Days**															**Cohort 5 Only Washout**	
	D29	D30	D31	D32	D33 (+1D)	D34	D35	D36 (+2D)	D37	D38	D39	D40	D41	D42	D43 (+2D)	D50 (+2D)	D57 (+2D)
Assess IP and Stool compliance	X							X							X		X
Stool Questionnaire	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Daily Dosing 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			
Hematology & Chemistry testing	X														X		X
Immunology testing	X							X							X		X
Stool (metagenomics)	X				X			X							X	X	X
Stool (metabolomics)	X							X							X		X
Stool (microbiology)	X							X							X		X
Fecal pH (using nitrazine paper)	X							X							X		X

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STATISTICAL ANALYSIS PLAN

Protocol: 20-CT-001

Dietary Study of Human Milk Oligosaccharides Concentrate PBCLN-005 with and without Bifidobacterium infantis in Healthy Adult Volunteers

SAP Version: 1.0 **Date of Final Change: 4/18/2023**

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Revision History

Version #	Date Effective	Page/Section/ Paragraph	Change From:	Change To:

TABLE OF CONTENTS

1 LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

Abbreviation	Definition
AE	Adverse event
ALT	Alanine transaminase
AST	Aspartate transaminase
BUN	Blood urea nitrogen
BMI	Body-mass index
CRF	Case report form
CRO	Clinical research organization
DSMB	Data and safety monitoring board
EIA	Enzyme immunoassay
FDA	Food and Drug Administration
GRAS	Generally regarded as safe
GDH	Glutamate dehydrogenase
GCP	Good clinical practice
HIPAA	Health Insurance Portability and Accountability Act
HMO	Human milk oligosaccharide
ID	Identification number
IEC	Independent ethics committee
IP	Investigational product
ISDA	Infectious Disease Society of America
IRB	Institutional review board
ICH	International Conference on Harmonization
NDA	Investigational New Drug
LDH	Lactate dehydrogenase
LNnT	Lacto-N-neotetraose
NGS	Next-generation sequencing
NAAT	Nucleic acid amplification tests
OTU	Operational taxonomic unit
OTC	Over the counter

Abbreviation	Definition
PO	Per os
RTF	Ready-to-feed
SAE	Serious adverse event
SHEA	Society of Hospital Epidemiologists of America
VRE	Vancomycin-resistant Enterococci

2 SCOPE OF THE STATISTICAL ANALYSIS PLAN

This version of the Statistical Analysis Plan (SAP) will focus on the safety analyses described in the protocol. These analyses will be included in an initial abbreviated safety report. The details of the analysis of primary and secondary endpoints are included here but will be expanded upon in an amended SAP for the full clinical study report.

3 INTRODUCTION

3.1 PREFACE

Human milk oligosaccharide (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.[1,2,4] Approximately 200 different HMO structures have been identified, although any particular mother will only produce a subset of those. Studies have shown that HMOs can alter the gut microbiome by favoring the growth of certain organisms as well as, perhaps, by inhibiting others.[4] It has also been demonstrated that HMOs can act as "decoy" receptors for a variety of pathogens and toxins.[4] They have also been shown, in vitro, to directly interact with certain components of the adaptive immune system.[4]

As a result of the aforementioned activities, it is 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 HMOs 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 might include conditions as disparate as inflammatory bowel disease and stem cell transplantation.

We hypothesize that *B. longum* subspecies *infantis* (*B. infantis*) might be a particularly beneficial organism to expand in the microbiome when managing such diseases in combination with HMOs. 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.[7] These dietary effects are postulated to be due to metabolism of HMOs by *B. infantis*, as infants receiving human milk in the absence of *B. infantis* don't show these effects. Importantly, *B. infantis* is not observed as a constituent of the human adult gut microbiota so it may therefore be necessary to administer both HMOs and *B. infantis* to obtain the full beneficial impact of HMOs feeding in adults.

Prolacta Bioscience manufactures human milk-based products from donated breast milk. In the course of the manufacture of human milk-based human milk fortifier, skim breast milk is ultra-filtered in order to concentrate proteins. Investigation of the permeate produced through this process demonstrated that human milk oligosaccharides were present in amounts comparable to those found in raw breast milk. Moreover, as the starting material consists of breast milk pooled from between 100 and 200 mothers, the full range of HMOs could be found. Subsequently, a method was developed to allow for the production of material with elevated concentrations of HMOs which contains little to no residual lactose through the introduction of lactase which is then removed.

An unconcentrated, pasteurized form of HMO material containing a proportional amount of lactose has already been used in an NIH sponsored clinical trial in premature infants with no reported ill effects.[v] Prolacta Bioscience has also completed a healthy volunteer study using the same HMO material as in this trial (with no addition of *B. infantis*) which showed good tolerability. No treatment emergent adverse effects were reported over the course of the trial.[6]

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).[8]

3.1 THE PURPOSE OF THE ANALYSES

The primary goal of this study is to evaluate the synergy of PBCLN-005 in varying doses with a fixed dose of *B. infantis*, compared with either component of the IP given alone. Synergy is defined as the enhanced gastrointestinal colonization of *B. infantis* upon feeding with HMO feeding. Secondary endpoints will measure changes in the gut microbiome, fecal metabolome and the subject's immune status as measured by systemic markers. We hypothesize that the combination will result in greater proliferation of *B. infantis* than was seen with the HMO concentrate alone.

Another objective is to understand the role of the low pH of the stomach in colonization of *B. infantis*. For most non-spore forming gut bacteria, the stomach is a barrier to colonization since acid exposure can reduce bacterial titer by 1,000- to 1,000,000-fold. While most subjects in the study will take an over-the-counter proton pump inhibitor (PPI; Zegerid: omeprazole plus sodium bicarbonate) to transiently reduce the production of gastric acid prior to *B. infantis* ingestion, one study group will ingest *B. infantis* without PPI pretreatment. We hypothesize that PPI pretreatment is necessary to observe *B. infantis* colonization.

We also hypothesize that ingestion of PBCLN-005 along with the *B. infantis* will result in a more marked change in immune markers than was seen in the previous study in healthy adults. We will, therefore, evaluate changes in circulating cytokine and growth factor levels following the ingestion of the study compared to baseline levels and between dose groups.

3.2 STUDY ENDPOINTS

3.2.1 Primary

The primary endpoint will be changes in the level of B. infantis from baseline evaluated by quantitative PCR using primers for species and strain. 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.

3.2.2 Secondary

- 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 taxonomy units (OTUs), relative abundance of various taxa, diversity (alpha and beta) and stability of communities and functional metabolomic changes.
- 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.
- Blood parameters, such as cytokine levels at study days -1, 8, 15 and 2 except for Cohort 5 who will also be evaluated on days 36, 43 and 57
- Determination of the need for protection of B. infantis from stomach acid in order for engraftment of the organism into the gut microbiome to occur.

3.2.3 Safety

- Total exposure to the test product will be calculated.
- Adverse events will be summarized by severity and relationship to study product.
- The laboratory evaluations listed below will be evaluated for clinical lab and medical safety at the following visits: Screening, Day 1, Day 15, Day 29, and, for Cohort 5, Day 43 and 57.
    - CBC with differential and platelets

- Alkaline phosphatase, alanine transaminase (ALT), aspartate transaminase (AST), lactate dehydrogenase (LDH), total and conjugated bilirubin, albumin and total protein for liver function
- Electrolytes (Na, K, Cl, and HCO_3)
- Glucose, total calcium, magnesium, phosphate, creatinine, and blood urea nitrogen (BUN) for renal function.

- Vital signs (heart rate, respiratory rate, temperature, and blood press are collected at the following visits: Screening, Day 1, Day 8, Day 15, Day 29, and for Cohort 5, Day 36, Day 43, and Day 57.
  - Heart rate
  - Respiratory rate
  - Temperature
  - Blood pressure

4 STUDY METHODS

4.1 STUDY DESIGN

The study is an unblinded six cohort multi-dose trial using 10 subjects per cohort for a total of 60 healthy adult volunteers ages 18-75 in order to evaluate the effects of the use of HMO Concentrate (PBCLN-005) and B. infantis on the human microbiome.

Six separate cohorts of both male and female study subjects will receive some form of the IP. In Cohort 1, the IP will consist of the standard dose of B. infantis alone. In cohort two, the IP will consist of only the PBCLN-005. In Cohort groups 3, 4, 5, and 6, the IP will consist of varying dose levels of PBCLN-005 together with B. infantis, with each cohort getting a single dose level of HMO. In Cohort groups 3, 4, 5, and 6, the IP will contain a standard dose of B. infantis.

Table 1 below summarizes the cohort dosing.

Table 1 Dosing cohorts

Cohort	Target HMO Amount (days 1-14)	PPI (days 1-7)	*B. infantis* (days 1-7)
1	None	Yes, 20 mg	Yes, one sachet
2	18g	Yes, 20 mg	None
3	4.5g	Yes, 20 mg	Yes, one sachet
4	9g	Yes, 20 mg	Yes, one sachet
5A	18g	Yes, 20 mg	Yes, one sachet
6	18g	No	Yes, one sachet

4.2 INCLUSION/EXCLUSION CRITERIA AND THE GENERAL PATIENT POPULATION

4.2.1 Inclusion Criteria

- Healthy adults between the ages of 18-75 years
- Willingness to complete study specific questionnaires
- Willingness to complete journal to record IP dosing times
- Willingness to complete all study procedures and clinic visits, and provide required samples
- Able to provide stool samples on specific study days
- Willingness to collect and process stool samples at home and transport stool samples to clinic
- Bristol Stool Score of 1 through 5 at time of screening
- Able to store up to 9 days of IP in refrigerator
- 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 PBCLN-005, 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
- Provides informed consent

4.2.2 Exclusion Criteria

- Women who are pregnant or breastfeeding, or intend to become pregnant during the course of this study
- Subjects with allergy to, or other reason for contraindication of the PPI chosen for this study.
- Subjects with history of lactose intolerance
- Subjects who are on a PPI regimen
- Subject who has taken a probiotic during the previous 30 days, or intends to take a probiotic during the study
- Subject who has taken antibiotics within 120 days
- Alcohol or drug abuse during the last 12 months, including passing a screen for drugs of abuse at screening
- Unstable medical condition, in the opinion of the investigator
- Clinically significant abnormal laboratory test results at screening
- Subjects who are unable or unwilling to provide stool samples on a regular basis

- 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.
- Carriage of B. infantis prior to study start, as determined by qPCR of stool
- Bristol Stool Score of 6 or 7 at time of screening

4.3 RANDOMIZATION AND BLINDING

The study will not utilize randomization or blinding.

4.4 SCHEDULE OF EVENTS

The tables below give the schedule of events for each cohort.

Schedule of Events Table
Cohort 1

Protocol Number 20-CT-001																		
Schedule of Events For Cohort 1 (Day 0 through Day 29)																		
Events	**Cohort 1 Screening**	**Cohort 1 Dosing Days**															**Cohort 1 Washout**	
	D0 (≤ 90 days prior to D1)	D1	D2	D3	D4	D5 (+1D)	D6	D7	D8 (+2D)	D9	D10	D11	D12	D13	D14	D15 (+2D)	D22 (+2D)	D29 (+2D)
Clinic visit	X	X							X							X		X
Final Visit																		X
Informed consent	X																	
Review IE Criteria	X	X																
Medical History	X																	
Physical Exam	X																	X
Demographics (age, gender, ethnicity and race	X																	
Vital Signs (Temp, BP, pulse, RR)	X	X							X							X		X
Height, weight and BMI	X																	
Prior & Con Meds	X	X							X							X		X
AEs		X							X							X		X

Cohort 1 (Cont.)

Protocol Number 20-CT-001																		
Schedule of Events For Cohort 1 (Day 0 through Day 29)																		
Events	**Cohort 1 Screening**	**Cohort 1 Dosing Days**															**Cohort 1 Washout**	
	D0 (≤ 90 days prior to D1)	D1	D2	D3	D4	D5 (+1D)	D6	D7	D8 (+2D)	D9	D10	D11	D12	D13	D14	D15 (+2D)	D22 (+2D)	D29 (+2D)
Randomization		X																
Dispense IP: B. infantis (≥8x107 cfu/d) and aqueous dilute		X																
Dosing: *B. infantis* (≥8x10^7 cfu/d)		X	X	X	X	X	X	X										
Assess IP and Stool compliance									X									X
Stool Questionnaires		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Daily Dosing and Flavor Questionnaire		X	X	X	X	X	X	X										
Patient Phone Calls			X	X	X	X	X	X										

Cohort 1 (Cont.)

Protocol Number 20-CT-001																		
Schedule of Events For Cohort 1 (Day 0 through Day 29)																		
Events	**Cohort 1 Screening**	**Cohort 1 Dosing Days**															**Cohort 1 Washout**	
	D0 (≤ 90 days prior to D1)	D1	D2	D3	D4	D5 (+1D)	D6	D7	D8 (+2D)	D9	D10	D11	D12	D13	D14	D15 (+2D)	D22 (+2D)	D29 (+2D)
Urine Drug Screen Test	X																	
Urine Pregnancy Test (Females of child-bearing potential)	X																	
Hematology & Chemistry testing	X	X														X		X
Immunology testing (Screening samples will not be evaluated for eligibility)	X	X							X							X		X
Stool (metagenomics) (Screening samples will not be evaluated for eligibility)	X	X				X			X							X	X	X
Stool (metabolomics) (Screening samples will not be evaluated for eligibility)	X	X							X							X		X
Stool (microbiology)	X	X							X							X		X
Fecal pH (using nitrazine paper)	X	X							X							X		X

Schedule of Events Table
Cohorts 2, 3, 4, and 6

Protocol Number 20-CT-001																		
Schedule of Events For Cohorts 2, 3, 4, & 6 (Day 0 through Day 29)																		
Events	**Cohorts 2, 3, 4, & 6 Screening**	**Cohorts 2, 3, 4, & 6 Dosing Days**															**Cohorts 2, 3, 4, & 6 Washout**	
	D0 (≤ 90 days prior to D1)	D1	D2	D3	D4	D5 (+1D)	D6	D7	D8 (+2D)	D9	D10	D11	D12	D13	D14	D15 (+2D)	D22 (+2D)	D29 (+2D)
Clinic visit	X	X							X							X		X
Final Visit																		X
Informed consent	X																	
Review IE Criteria	X	X																
Medical History	X																	
Physical Exam	X																	X
Demographics (age, gender, ethnicity and race	X																	
Vital Signs (Temp, BP, pulse, RR)	X	X							X							X		X
Height, weight and BMI	X																	
Prior & Con Meds	X	X							X							X		X
AEs		X							X							X		X

Cohorts 2, 3, 4, and 6 (cont.)

Protocol Number 20-CT-001																		
Schedule of Events For Cohorts 2, 3, 4 & 6 (Day 0 through Day 29)																		
Events	**Cohorts 2, 3, 4, & 6 Screening**	**Cohorts 2, 3, 4, & 6 Dosing Days**															**Cohorts 2, 3, 4, & 6 Washout**	
	D0 (≤ 90 days prior to D1)	D1	D2	D3	D4	D5 (+1D)	D6	D7	D8 (+2D)	D9	D10	D11	D12	D13	D14	D15 (+2D)	D22 (+2D)	D29 (+2D)
Randomization		X																
Dispense IP:, B. infantis (≥8x107 cfu/d) (except Cohort 2) (Note: On D1 dispense 9-day supply of PBCLN-005. On D8 dispense 5-day supply of PBCLN-005)		X							X									
Dosing: *B. infantis* (≥8x10^7 cfu/d) (All Cohorts except Cohort 2)		X	X	X	X	X	X	X										
Dosing: PBCLN-005 1 bottle po bid (Target 4.5g, 9g or 18g)		X	X	X	X	X	X	X	X	X	X	X	X	X	X			
Assess IP and Stool compliance		X							X							X		X
Stool Questionnaire		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Daily Dosing 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			

Cohorts 2, 3, 4 & 6 (cont.)

Protocol Number 20-CT-001																		
Schedule of Events For Cohorts 2, 3, 4, & 6 (Day 0 through Day 29)																		
Events	**Cohorts 2, 3, 4, & 6 Screening**	**Cohorts 2, 3, 4, & 6 Dosing Days**															**Cohorts 2, 3, 4, & 6 Washout**	
	D0 (≤ 90 days prior to D1)	D1	D2	D3	D4	D5 (+1D)	D6	D7	D8 (+2D)	D9	D10	D11	D12	D13	D14	D15 (+2D)	D22 (+2D)	D29 (+2D)
Urine Drug Screen Test	X																	
Urine Pregnancy Test (Females of child-bearing potential)	X																	
Hematology & Chemistry testing	X	X														X		X
Immunology testing (Screening samples will not be evaluated for eligibility)	X	X							X							X		X
Stool (metagenomics) (Screening samples will not be evaluated for eligibility)	X	X				X			X							X	X	X
Stool (metabolomics) (Screening samples will not be evaluated for eligibility)	X	X							X							X		X
Stool (microbiology)	X	X							X							X		X
Fecal pH (using nitrazine paper)	X	X							X							X		X

Protocol Number 20-CT-001																	
Schedule of Events For Cohort 5 (Day 0 through Day 22)																	
Events	**Cohort 5 Screening**	**Cohort 5 1st Dosing Course**															**Cohort 5 1st Washout**
	D0 (≤ 90 days prior to D1)	D1	D2	D3	D4	D5 (+1D)	D6	D7	D8 (+2D)	D9	D10	D11	D12	D13	D14	D15 (+2D)	D22 (+2D)
Urine Drug Screen Test	X																
Urine Pregnancy Test (Females of child-bearing potential)	X																
Hematology & Chemistry testing	X	X														X	
Immunology testing (Screening samples will not be evaluated for eligibility)	X	X							X							X	
Stool (metagenomics) (Screening samples will not be evaluated for eligibility)	X	X				X			X							X	X
Stool (metabolomics) (Screening samples will not be evaluated for eligibility)	X	X							X							X	
Stool (microbiology)	X	X							X							X	
Fecal pH (using nitrazine paper)	X	X							X							X	

Cohort 5 (cont.)

Protocol Number 20-CT-001																	
Schedule of Events For Cohorts 5 Only (Day 29 through Day 57)																	
Events	**Cohort 5 2nd Dosing Course**															**Cohort 5 2nd Washout Period**	
	D29	D30	D31	D32	D33 (+1D)	D34	D35	D36 (+2D)	D37	D38	D39	D40	D41	D42	D43 (+2D)	D50 (+2D)	D57 (+2D)
Clinic visit	X							X							X		X
Final Visit																	X
Physical Exam																	X
Vital Signs (Temp, BP, pulse, RR)	X							X							X		X
Prior & Con Meds	X							X							X		X
AEs	X							X							X		X
Dispense IP: PPI(if applicable)[1] , B. infantis (≥8x107 cfu/d) (Note: On D29 dispense 9-day supply of PBCLN-005. On D36 dispense 5-day supply of PBCLN-005)	X[1]							X[1]									
Dosing: PPI[1]	X[1]	X[1]	X[1]	X[1]	X[1]	X[1]	X[1]										
Dosing: *B. infantis* (≥8x10^7 cfu/d)	X	X	X	X	X	X	X										
Dosing: PBCLN-005 (Target 18g)	X	X	X	X	X	X	X	X	X	X	X	X	X	X			

Cohort 5 (cont.)

Protocol Number 20-CT-001																	
Schedule of Events For Cohorts 5 (Day 29 through Day 57)																	
Events	**Cohort 5 2nd Dosing Days**															**Cohort 5 Only Washout**	
	D29	D30	D31	D32	D33 (+1D)	D34	D35	D36 (+2D)	D37	D38	D39	D40	D41	D42	D43 (+2D)	D50 (+2D)	D57 (+2D)
Assess IP and Stool compliance	X							X							X		X
Stool Questionnaire	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Daily Dosing 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			
Hematology & Chemistry testing	X														X		X
Immunology testing	X							X							X		X
Stool (metagenomics)	X				X			X							X	X	X
Stool (metabolomics)	X							X							X		X
Stool (microbiology)	X							X							X		X
Fecal pH (using nitrazine paper)	X							X							X		X

4.5 SAMPLE SIZE

Six groups of 10 subjects will be included in this study for a total sample size of 60. The sample size was not determined statistically, but rather represents a typical number for an exploratory study of healthy volunteers.

4.6 TIMING OF ANALYSES

There will be a single analysis of the data at the conclusion of the enrolment of the subjects and the collection of the study endpoints

4.7 ANALYSIS POPULATIONS

Since this study is a safety study, the only analytical population will be individuals who receive at least one dose of either active drug or placebo.

4.8 MISSING DATA

Investigators should make every effort to ensure that loss to follow-up is kept to a minimum and that all data are collected for all subjects at all time points. However, because this is a Phase 1 safety study, no data imputation methods will be used to account for missing data.

4.9 INTERIM ANALYSES AND DATA MONITORING

There are no interim analyses conducted during this study.

5 SUMMARY OF STUDY DATA

All continuous variables will be summarized using the following descriptive statistics: n (non-missing sample size), mean, standard deviation, median, maximum and minimum. The frequency and percentages (based on the non-missing sample size) of observed levels will be reported for all categorical measures. In general, all data will be listed, sorted by cohort and subject, and when appropriate by visit within subjects. All summary tables will be structured with a column for each cohort.

5.1 SUBJECT DISPOSITION

A tabulation of subject disposition will be presented by cohort and overall, including the number screened, the number dosed at each level, the number for primary analysis, the number that withdrew prior to completing therapy, and reasons for withdrawal.

5.2 PROTOCOL DEVIATIONS

Major protocol deviations will be determined at the end of the study when all data have been entered into the clinical database. Protocol violations will be listed by patient.

5.3 DEMOGRAPHIC AND BASELINE VARIABLES

Patient distribution across demographic and baseline characteristics will be tabulated and presented by dose group and overall.

The following demographic and baseline characteristics will be evaluated:

1) Age (calculated as [date of study entry-date of birth]/365.25)
2) Gender
3) Ethnicity
4) Race
5) Physical exam
    - Height
    - Weight
    - BMI
    - Body systems (Normal/Abnormal – clinically significant/Abnormal – not clinically significant/Not Done)
        - General Appearance
        - HEENT
        - Heart and cardiovascular
        - Dermatologic
        - Gastrointestinal
        - Musculoskeletal
        - Lymphatic
        - Neurological
        - Respiratory
6) Vital signs
    - Blood pressure
    - Heart rate
    - Respiratory rate

- Temperature

7) Baseline medical history – MedDRA coded and tabulated by system organ class and preferred term

8) Baseline laboratory assessments

6 PRIMARY ENDPOINT ANALYSES

The primary endpoint will be changes in the level of B. infantis from baseline evaluated by quantitative PCR using primers for species and strain.

7 SECONDARY ENDPOINT ANALYSES

Since this is an initial study of the use of PBCLN-005 in a clinical population, it is, by definition, hypothesis-generating. Therefore, all statistical analyses will be descriptive for the evaluation of the data between and within dose groups and the placebo group.

7.1 CHANGES IN STOOL MICROBIOTA

7.1.1 Fecal Microbial Diversity

This will include changes in the diversity, composition, and microbial taxa abundance. Microbial evenness and richness will be evaluated across all cohorts.

7.2 CHANGES IN VIABILITY OF PROTEOBACTERIA AND ENTEROCOCCUS

7.3 CHANGES IN BLOOD PARAMETERS

7.4 EVALUATION OF THE USE OF PROTON PUMP INHIBITORS FOR PROTECTION OF B. INFANTIS

8 SAFETY EVALUATION

The safety analyses of exposure, AEs and laboratory parameters will include descriptive statistics and will be summarized separately by dose group and overall. Summaries of AEs will be generated by type (AE or SAE), body system and preferred term, severity, and relationship to study product.

8.1 ADVERSE EVENTS

All reported AEs, will be listed, documenting the course, outcome, severity, and causality to study drug. Verbatim terms on CRFs will be mapped to preferred terms and related system organ class using the Medical Dictionary for Regulatory Activities (MedDRA).

Incidence rates of AEs and the proportion of subjects prematurely withdrawn from the study due to AEs will be shown for all dose groups. Incidence rates will also be displayed based on severity and relationship to study drug. AEs with a relationship of "possibly" or "probably" related will be considered by the Sponsor as "related" to the study drug. Events assessed as "unrelated", "unlikely" related, or where the relationship was not reported will be considered by the Sponsor as "not related" to the study drug. The incidence of SAEs will be provided for each phase. All incidence rates will be categorized and displayed by system organ class and preferred term.

8.2 CLINICAL LABORATORY EVALUATIONS

The laboratory evaluations listed below will be evaluated for clinical lab and medical safety at the following visits: Screening, Day 1, Day 15, and Day 29. For Cohort 5 only, add Day 43, Day 57.

- CBC with differential and platelets
- Alkaline phosphatase, alanine transaminase (ALT), aspartate transaminase (AST), lactate dehydrogenase (LDH), total and conjugated bilirubin, albumin and total protein for liver function
- Electrolytes (Na, K, Cl, and HCO_3)
- Glucose, total calcium, magnesium, phosphate, creatinine, and blood urea nitrogen (BUN) for renal function.

All laboratory values will be summarized by descriptive statistics; mean ± SD, median ± interquartile range along with counts and percentages for categorical data.

8.3 VITAL SIGNS

Vital signs (heart rate, respiratory rate, temperature, and blood pressure) are collected at the following visits: Screening, Day 1, Day 8, Day 15, and Day 29. For Cohort 5 only, add Day 36, Day 43, Day 57.

Descriptive statistical summaries will be presented by dose group and visit.

9 TECHNICAL DETAILS

All analyses will be conducted using SAS Version 9.4. MedDRA Version 24.0 will be used to code adverse events and medical history conditions.

10 CHANGES IN CONDUCT OF STUDY OR TO PLANNED ANALYSES FROM PROTOCOL

Deviations from the statistical analyses outlined in this plan will be indicated; any further modifications would be noted in the final statistical analysis.

11 REFERENCES

[i] Barile D, Lebrilla DBC, German B, Rechtman DJ, and Lee ML. Oligosaccharide prebiotics present in a breast milk based human milk fortifier. Presented at Hot Topics in Neonatology. Washington DC December 2008

[ii] Barile D, German B, Lee ML, and Rechtman DJ. Potential Novel Source for Human Oligosaccharides. Presented at American Academy of Pediatrics National Conference and Exhibit, Washington DC 2009.

[iii] Ninonuevo M., Park Y., YIN H., Zhang J., Ward R.E., Clowers B.H., German J.B., Freeman S.L., Killeen K., Grimm R., and Lebrilla C.B.(2006) A strategy to annotate the human milk glycome. J.Agric. Food Chem. 54: 7471-7480]

[iv] Smilowitz JT, Lebrilla CB, Mills DA, German JB, and Freeman SL. Breast Milk Oligosaccharides: Structure-Function Relationships in the Neonate. Annu Rev Nutr. 2014 ; 34: 143–169.

[v] Underwood MA, Kalanetra KM, Bokulich NA et al. Prebiotic oligosaccharides in premature infants. JPGN 2014;58: 352–360

[vi] Prolacta Bioscience unpublished data on file

[7] https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5450358/

[8] https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6114170/

12 LISTING OF TABLES, LISTINGS, AND FIGURES

TABLES

Number	Table Title
14.1.2	Summary of Demographics and Baseline Characteristics

14.1.3	Summary of Medical History
14.3.4.1	Summary of Hematology by Dose Group and Visit
14.3.4.2	Summary of Electrolytes/Renal by Dose Group and Visit
14.3.4.3	Summary of Liver Serum Analyses by Dose Group and Visit
14.3.4.4	Summary of Physical Exams by Dose Group and Visit
14.3.4.5	Summary of Vital Signs by Dose Group and Visit
14.3.4.6	Summary of Prior and Concomitant Medications
14.3.1.2	Number and Percentage of Subjects with Adverse Events by Treatment Group, System Organ Class and Preferred Term
14.3.1.4	Number and Percentage of Subjects with Adverse Events by Treatment Group, System Organ Class, Preferred Term and Relationship to Treatment
14.3.1.5	Number and Percentage of Subjects with Adverse Events Resulting in Withdrawal from Treatment by Treatment Group, System Organ Class, and Preferred Term
14.3.1.6	Number and Percentage of Subjects with Adverse Events by Treatment Group, System Organ Class, Preferred Term, and Severity

LISTINGS

Number	Listing Title
16.2.1.1	Subject Eligibility
16.2.2.1	Subject Disposition
16.2.2	Protocol Deviation
16.2.4.1	Demographics
16.2.4.2	Medical History
16.2.4.3	Prior and Concomitant Medications
16.2.7	Adverse Events
16.2.8.1	Laboratory Results - Hematology
16.2.8.2	Laboratory Results – Serum Chemistry
16.2.8.3	Laboratory Results – Urinalysis (Drug Screen)
16.2.8.4	Laboratory Results – Pregnancy Test
16.2.8.5	Vital Signs
16.2.8.6	Physical Exams