Mass General Brigham

Institutional Review Board
Intervention/Interaction Detailed Protocol

Principal Investigator: **Qian Yuan, MD, PhD**

Project Title: The **RESTORE** Study: Restoring gut health with *B. infantis* INF108F in Infants with Food Protein Induced Allergic Proctocolitis

Version Date: 08/01/2023

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IND Sponsor: N/A

PRINCIPAL INVESTIGATOR	**CO-INVESTIGATOR(S)**
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INVESTIGATOR SIGNATURE PAGE	
Protocol	**Version/Date:**
pending	0.1 / June 01, 2023

IND Number	**Principal Investigator:**
N/A	Qian Yuan, MD, PhD

Title: The RESTORE Study: Restoring gut health with *B. infantis* INF108F in infants with Food Protein Induced Allergic Proctocolitis

Study Sponsor: Massachusetts General Hospital and Infinant Health

INSTRUCTIONS: The Principal Investigator will print, sign, and date at the indicated location below. The original signature page will be kept by the PI and a copy will be sent to Infinant Health. After signature, the original will be kept by:

Jannat Gill
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I confirm that I have read the above protocol in the latest version. I understand it, and I will work according to the principles of good clinical practice (GCP) as described in the US Code of Federal Regulations (CFR) 45 CFR part 46 and 21 CFR parts 50, 56, and 312, and in the International Conference on Harmonization (ICH) document Guidance for Industry: E6 Good Clinical Practice: Consolidated Guidance dated April 1996. Further, I will conduct the study in keeping with local legal and regulatory requirements.

As the principal investigator, I agree to carry out the study by the criteria written in the protocol and understand that changes to this protocol will only be made by mutual consensus with Infinant Health.

Qian Yuan, MD, PhD

_____

Principal Investigator (Print)

_____

Principal Investigator (Signature) Date

SYNOPSIS

Title	The RESTORE Study: Restoring gut health with *B. infantis* INF108F in Infants with Food Protein Induced Allergic Proctocolitis
Short Title	The RESTORE Study
Rationale	Food Protein Induced Allergic Protocolitis (FPIAP) is an early form of food allergy in infancy. It is thought be related to the dysbiosis of the infant gut microbiome. *Bifidobacterium longum* subspecies (subsp.) *infantis* (*B. infantis*) is one of the special bacterial strains which is adapted to metabolize human milk oligosaccharides (HMOs) from breast milk. Lack of bifidobacteria, and in particular depletion of genes required for HMO utilization from the metagenome, is associated with systemic inflammation and immune dysregulation early in life. In breastfed infants, supplementation with *B. infantis* has been shown to alter the gut microbiome composition and correct the dysbiosis associated with immune dysfunction.
Clinical Phase	Post-market, Food for Special Dietary Use with GRAS status
Mechanistic Study	Yes
Study Sponsor	Massachusetts General Hospital and Infinant Health
Principal Investigator	Qian Yuan, MD, PhD
Participating Site(s)	Food Allergy Center @ Massachusetts General Hospital
Accrual Objective	50 randomized
Investigational Product / Intervention	*B. infantis* INF108F versus placebo
Study Objectives	To evaluate the ability of *B. infantis* INF108F to promote a change in the gut microbiome composition of infants with FPIAP over a 4-week period. Secondary objectives are to evaluate changes to clinical symptoms of FPIAP and additional stool biomarkers.
Study Design	This is a single-center, randomized, double-blind, placebo-controlled trial with two arms evaluating *B. infantis* INF108F (Food for Special Dietary Use) in breastfed infants with FPIAP. The particular sub-species of *Bifidobacterium* used in this study has historically dominated the breastfed infant gut, however, it is currently missing from the gastrointestinal tract of most infants in the industrialized world. There is evidence to support that *B. infantis* INF108F supplementation may ameliorate symptoms associated with FPIAP by establishing and maintaining the necessary gut

	microbial composition to promote proper barrier and immune function in infants.
Study Duration	4 weeks

SYNOPSIS CONTINUED

Primary Endpoint	Changes to the gut microbiome composition (including *B. infantis* colonization) in infants with FPIAP over a 4-week period (*B. infantis* INF108F vs placebo)
Secondary Endpoints and Biomarkers / Exploratory Endpoints	**Secondary Endpoints:** • The percentage of patients testing negative for blood in stool by Study Day 7, 14 and 28 by hemoccult testing • The percentage of patients with no gross/visible blood in stool by Study Day 7, 14 and 28 by parental report • Changes to stool frequency by Study Day 7, 14 and 28 • Changes to clinical symptoms including GER, feeding difficulties, sleep disturbance and poor growth • The occurrence of supplement-related adverse events **Biomarkers / Exploratory Endpoints:** • Fecal calprotectin levels • Fecal pH levels • Fecal metabolomics • Markers of inflammation and enteric cytokines including, but not limited to, IL-1, IL-8 and TNF-alpha in stool • Levels of eosinophil-related granular proteins in stool • Intestinal permeability/integrity markers including, but not limited to, lipocalin (NGAL)

Inclusion Criteria	• Infants, male or female, of all ethnic/racial groups, with a gestational period of 37 to 42 weeks • Infants aged 1-90 days old at the time of enrollment with documented FPIAP with either gross blood or microscopic blood in stools, without other possible causes • Infants must be exclusively breastfed or at least half of oral intake is from breastfeeding or from expressed breast milk • A willing parent or legal guardian will sign the consent form either electronically or with a wet ink signature

SYNOPSIS CONTINUED

Exclusion Criteria	• Infants born earlier than 37 weeks of gestation • Infants who are exclusively-formula fed or less than half of oral intake is from breastfeeding or from expressed breast milk at the time of enrollment • Infants born with medical complications (i.e., neurological, cerebral palsy, confirmed food allergies) • Diagnosis of other severe or complicating medical problems, including autoimmune or chronic immune inflammatory conditions, gastrointestinal inflammatory conditions, or renal insufficiency • History of abdominal surgery or congenital abnormalities of the GI tract, the cardiovascular system, the pulmonary system or the renal system • Antibiotic use (oral or systemic) within 7 days prior to enrollment • Parents' intent to feed non-study probiotics or solid food to their infant at any time during the study • Infants who have consumed any *B. infantis*-containing probiotics since birth • Primary Immune Deficiency • Maternal use of probiotics containing *B. infantis* after the baby's birth and/or intent to use probiotics containing *B. infantis* at any time throughout the study • Past or current medical problems or findings from physical examination or laboratory testing that are not listed above, which, in the opinion of the investigator, may pose additional risks from participation in the study, may interfere with the participant's ability

	to comply with study requirements or that may impact the quality or interpretation of the data obtained from the study
Study Procedures	Physical examination, stool collection, questionnaire administration
Statistical Considerations	20 FPIAP infants in each group, *B. infantis* INF108F versus placebo. Approximately 100 infants will be recruited and screened for the study to obtain a sample size of 50 participants for randomization, assuming a 50 percent screen fail rate. Forty (40) participants are expected to complete the study assuming a 20 percent drop-out rate. This sample size is based on a literature review of randomized clinical trials and the anticipated changes to the microbiome based on previous studies of *B. infantis*. Analysis on primary and secondary objectives will be completed based on the mITT population and the PP population; analysis on the exploratory objectives will be completed based on the mITT population.

Contents

Glossary of Abbreviations / Definitions

AE	Adverse Event
AR	Adverse Reaction
CFR	Code of Federal Regulations
CFU	Colony Forming Units
CMO	Contract Manufacturing Organization
CRA	Clinical Research Associate
CRC	Clinical Research Center
CRF	Case Report Form
CTCAE	Common Terminology Criteria for Adverse Events
DNA	Deoxyribonucleic Acid
EC	Ethics Committee
EGID	Eosinophilic Gastrointestinal Disorder
eIC	Electronic Informed Consent
EoE	Eosinophilic esophagitis
FDA	Food and Drug Administration
FPIAP	Food Protein Induced Allergic Proctocolitis
FPIES	Food Protein Induced Enterocolitis Syndrome
FSDU	Foods for Special Dietary Use
cGCP	Current Good Clinical Practice
GCP	Good Clinical Practice
GERD	Gastroesophageal Reflux Disease
GI	Gastrointestinal
GLP	Good Laboratory Practices
GMAP	Gastrointestinal Microbiome Allergic Proctocolitis
GMP	Good Manufacturing Practices
GRAS	Generally Regarded as Safe
HIPAA	Health Insurance Portability and Accountability Act
HMO	Human Milk Oligosaccharide
ICH	International Conference on Harmonization
IRB	Institutional Review Board
ISM	Independent Safety Monitor
ITT	Intention to Treat
NIH	National Institutes of Health
PCR	Polymerase Chain Reaction

PHI	Protected Health Information
PI	Principal Investigator
QA	Quality Assurance
QC	Quality Control
RNA	Ribonucleic Acid
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SAR	Serious Adverse Reaction
SMC	Safety Monitoring Committee
SOP	Standard Operating Procedure
US	United States

1. BACKGROUND AND SIGNIFICANCE

1.1 Background

Oral tolerance is one of the fundamental mechanisms in preventing food allergies in humans. Breaching of oral tolerance may potentially lead to the development of food allergies (FA). Allergic food reactions can be divided into 2 categories: immunoglobulin E (IgE)-mediated and non-IgE-mediated FA. The hallmark of IgE-mediated FA is the potential of life-threatening anaphylaxis. The frequency of IgE-mediated FA in US children is about 6-8%.

The non-IgE-mediated GI FA includes several clinical conditions, food protein-induced allergic protocolitis (FPIAP), food protein induced enterocolitis syndrome (FPIES), eosinophilic esophagitis (EoE) and eosinophilic gastrointestinal disorder (EGID). FPIAP is likely the earliest manifestation of non-IgE-mediated FA in childhood, and perhaps the very first condition of breaching oral tolerance, as it occurs in early infancy (2 weeks to 2 months) (1). In a prospective birth cohort study (GMAP), we have demonstrated that a prevalence of 17% of infants with FPIAP typically present with mucusy bloody stools, GER, irritability and feeding intolerances (1), and have increased risk of developing IgE-mediated FA later in life (2). FPIAP is also a risk factor for developing EoE (3). Children with FPIAP often have eczema (1). These suggest a possible common mechanism in non-IgE-mediated and IgE-mediated conditions.

The human intestinal microbiome is found to play a key role in health and disease. Changes to the gut microbiota have been associated with a wide variety of diseases, including allergic diseases in human (4-9). It is known that the human gut microbiota undergoes rapid and dynamic changes during the first year of life (10-13). Many factors are known to influence the initial acquisition and subsequent composition of the gut microbiome in infants (11). These factors include mode of delivery, antibiotic exposure, presence of mother's milk and breastfeeding, social economic status and household living conditions (11). Additionally, prenatal factors and in-utero exposures to certain environments likely play a role in the acquisition and composition of the infant gut microbiota (11).

The gut microbiota plays a key role in the development of functional immune responses at all stages of development. The hygiene hypothesis suggests that changes in our microbial environment, including increased antibiotic usage and decreased environmental microbe exposure, are responsible for the rise of allergic and inflammatory diseases in the industrialized world. The human intestine is densely colonized by trillions of microbes that exist within an ecological network, interacting constantly with the human host to provide nutrient digestion and production of secondary metabolites, competitive exclusion of pathogenic species, and immune modulation. Dysbiosis refers to abnormal changes of the composition of the gut microbiota. The etiology of dysbiosis of the infant human gut microbiome is likely caused, at least in part, by epi-genetic factors over several generations (14). Intestinal dysbiosis has been suggested to contribute to a variety of inflammatory diseases, both within the gut and systemically (15). While food allergy patients do not share the severe bacterial imbalances observed in the intestines of inflammatory bowel disease or Clostridium difficile patients, studies suggest that they possess a microbiota that differs from that of healthy individuals. In a recent study, higher abundance of Ruminococcus gnavus and *Faecalibacteerium prausnitzii* and a depletion of *Bifidobacterium longum*, *Bactereioudes dorei*, *B. vulgatus* and fiber-degrading taxa were found in the gut microbiome of allergic children (16). The phylum Firmicutes, and specifically, bacteria from Clostridial Clusters IV and XIVa have been strongly associated with immunoregulatory responses in the intestine and have protective effects in models of food allergy and colitis (17-20). Numerous animal studies have established that bacteria can modulate susceptibility to food allergy, and that beneficial Clostridia and their metabolites are protective in these models (18, 21-23). Thus, modulation of the microbiome shows promise as a therapeutic intervention for food allergies.

1.2 Rationale for Selection of Study Population

In a recent study, Henrick and colleagues elegantly demonstrated that a lack of bifidobacteria, specifically depletion of genes required for human milk oligosaccharide (HMO) utilization from the metagenome, is associated with systemic inflammation and immune dysregulation early in life (24). In our GMAP birth cohort, dysbiosis of the gut microbiome has been shown in both FPIAP (44) and FPIES (Su et al, submitted).

Correction of the dysbiosis and restoration of the healthy gut microbiome in early infancy have great potential to alter abnormal immune responses, to prevent and treat non-IgE-mediated FA in infancy, and also to possibly prevent or alter the development of subsequent other non-IgE-mediated and IgE-mediated FA and allergic conditions.

A birth cohort study from Finland has demonstrated that loss of Bifidobacterium early in life has been associated with increased risk of developing autoimmunity (25). This has also been shown in atopic wheezing in another cohort in rural Ecuador (26). Studies by Henrick and colleagues (27) and Rhoads and colleagues (28) have identified an association between the loss of Bifidobacterium in infants and enteric inflammation early in life.

Bifidobacterium longum subspecies *infantis* (*B. infantis*) is a specialized bacterium adapted to metabolize human milk oligosaccharides (HMOs). Human breast milk contains abundant HMOs that are not digestible by humans, as we lack the necessary glucosidases (29). Instead, the maternal energy spent to create such complex sugars is justified by providing a selective nutritional advantage to "beneficial" microbes specialized in metabolizing HMOs with evolutionarily important functions in newborns (24).

Introducing *B. infantis* has been successfully accomplished in strains such as INF108F (Infinant Health), which is able to stably and persistently colonize and remodel the intestinal microbiome of breastfed infants (30), leading to reduced fecal calprotectin, a marker of intestinal inflammation (27).

In breastfed infants given *Bifidobacterium infantis* INF108F, which expresses all HMO-utilization genes, intestinal T helper 2 (Th2) and Th17 cytokines were silenced and interferon γ (INF-γ) was induced (24). Fecal water from INF108F -supplemented infants contains abundant indolelactate and *B. infantis*-derived indole-3-lactic acid (ILA) upregulated immunoregulatory galectin-1 in Th2 and Th17 cells during polarization. These data provide a functional link between beneficial microbes and immunoregulation during the first months of life.

This protocol is designed to investigate the role of *B. infantis* in modulating the gut microbiome in infants with FPIAP and the changes from *B. infantis* on symptoms of FPIAP and stool biomarkers.

1.3 Investigational Product(s) / Intervention(s)

The active product that will be used in the proposed clinical trial is currently available commercially as a food supplement (Food for Special Dietary Use).

1.4 Rationale for Selection of Investigational Product(s) / Intervention(s) and Regimen

B. infantis INF108F has been used in other trials and has been shown to be safe and well-tolerated in infants (term and preterm) when supplemented daily for variable durations. Studies of this strain in breastfed infants

demonstrate a significant change to the gut microbiome composition within days of initiating the supplement. This change generally persists beyond the supplementation period if infants continue to receive breast milk as all or part of their diet.

1.5 Preclinical and Clinical Experience

1.5.1 Preclinical Studies and Regulatory Status

The investigational study product is a probiotic, *B. infantis* INF108F. *B. longum* subsp. *infantis* INF108F (Infinant Health) was selected for this study due to its demonstrated ability to utilize many types of oligosaccharides found in breast milk (i.e., HMOs), including lacto-N-tetraose, lacto-N-neo-tetraose, 2'-fucosyllactose, 3'-fucosyllactose, 3'-sialyllactose, and 6'-sialyllactose.

B. infantis INF108F was isolated from a healthy term breastfed infant. Its identity was confirmed by genetic sequencing of the organism by Infinant Health. Master stocks and working stocks were created and the strain is routinely tested for purity after fermentation. According to cGMP, the strain was re-isolated before creating the inoculum for the fermentation run.

B. infantis INF108F was produced by fermentation under cGMP conditions at a contract manufacturing organization (CMO). Following fermentation, the strain was centrifuged, freeze-dried, milled, and blended with lactose sourced from a Safe Quality Food (SQF) level 3 facility. The product was packaged by a second CMO into FDA-compliant single-serving nitrogen-flushed polyester-faced laminated sachets with an oxygen and moisture barrier following cGMP. Each sachet contains approximately 600 mg of the blended powder containing at least 8 x 10^9 CFU *B. infantis* INF108F.

By way of its unique combination with human breast milk and utilization of HMOs, *B. infantis* INF108F is designated as a "Foods for Special Dietary Use" (FSDU). The term "special dietary uses", as applied to food for man, is described in Chapter 1, Subchapter B, Part 105 of the Code of Federal Regulations, 21 CFR and defined in Part 105.3. It means foods supplying particular dietary needs which exist by reason of a physical, physiological, pathological or other condition, such as conditions of diseases, convalescence, pregnancy, lactation, allergic hypersensitivity to food, underweight and overweight. Included in the definition are uses for supplying particular dietary needs which exist by reason of age, including but not limited to the ages of infancy (not more than 12 months old) and childhood (more than 12 months but less than 12 years).

Within the FDA, the Office of Food Additive Safety oversees a process for evaluating the safety of food ingredients that have a history of use and can, by scientific consensus, be generally regarded as safe (GRAS). These food ingredients must complete a rigorous process of scientific and data review by independent qualified experts, who assess whether the product is safe under the conditions of its intended use. *B. infantis* INF108F gained GRAS status after successfully completing this process, with unanimous consensus by all independent qualified experts that this product is generally recognized as safe based upon the application of generally available and accepted scientific data, information, or methods (i.e., peer-reviewed scientific literature), and scientific principles for use in term infants.

GRAS status for *B. infantis* INF108F was obtained for a use level of up to 2.8 x 10^{10} colony forming units (CFU) per day. The current commercial serving size of *B. infantis* INF108F is 8.0 x 10^9 CFU/day. The study product control is a lactose placebo. The lactose ingredient was sourced from a SQF level 3 facility and packaged into FDA-compliant

single-serving nitrogen-flushed polyester-faced laminated sachets with an oxygen and moisture barrier following cGMP. Each sachet contains approximately 600 mg of lactose powder.

1.5.2 Clinical Studies Relevant for FPIAP

As mentioned earlier in Background section 1.1, Food Protein-induced Allergic Proctocolitis (FPIAP) is likely the very first manifestation of breaching oral tolerance in infancy (1, 31, 32, 33). It is one of the non-IgE mediated food allergies in infancy and early childhood (34, 35). Children with FPIAP have increased risk to develop IgE-mediated food allergies later in life (2, 33). Although the precise mechanism of FPIAP is still unclear, multifactorial causes including dysbiosis likely plays a role (36, 44).

Probiotic-based strategies have been used to treat allergic/atopic diseases in infancy and childhood (36, 37, 38, 39). Probiotics are now added to infant formulas by several formula makers with the intention to reduce atopic diseases in infancy; however, the specificity and efficacy vary.

B. infantis INF108F uses HMO-utilization genes to metabolize HMO in breastfed infants, suppresses Th2 and Th17 cytokines and promotes INF-g production, proving a functional link between beneficial microbes and immunoregulation during the first months of life (24).

2. SPECIFIC AIMS AND OBJECTIVES

2.1 Primary Objective

Assess the effect of modulating the gut microbiome by *B. infantis* INF108F in infants with FPIAP in early infancy.

2.2 Secondary and Exploratory Objective(s)

- Evaluate changes in FPIAP symptoms (blood in stool, stool frequency, GER, feeding difficulties, and sleep disturbance) from feeding *B. infantis* INF108F.
- Evaluate changes in enteric markers of inflammation and biochemistry from feeding *B. infantis* INF108F

3. GENERAL DESCRIPTION OF STUDY DESIGN

This single-center, double-blind, randomized and placebo-controlled clinical study will evaluate modulation of the intestinal microbiome in breastfed infants consuming a probiotic, *B. infantis* INF108F. The specific sub-species of bifidobacteria used in this study has historically dominated the breastfed infant gut; however, it is currently missing from the gastrointestinal tract of most infants in the industrialized world (40). There is evidence to support that *B. infantis* INF108F supplementation may ameliorate symptoms associated with FPIAP by establishing and maintaining the necessary gut microbial composition to promote proper barrier and immune function in infants

(41). We intend to enroll 100 infants for the study. The parents of each newborn will complete questionnaires and study logs and collect stool samples for the study. . Of the enrolled infants, we expect to randomize 50 in a 1:1 allocation to the following study arms:

- 8.0 x 10^9 CFU daily serving of *B. infantis* INF108F (25 infants)
- Lactose placebo (25 infants)

Study participants and staff will be blinded to the treatment assignments. Infants in the active cohort will receive a once-daily oral feeding of *B. infantis* INF108F (8.0 x 10^9 CFU) and infants in the control cohort will receive a lactose placebo (both supplements mixed with breast milk) for 14 consecutive days. Study product feeds will be initiated on Day 1 (after the baseline period) and will continue for 14 days. The *B. infantis* INF108F and placebo will both be provided as a powder in sachets and will be prepared by mixing with a small amount of breast milk. The mixture will be fed by syringe to ensure the entire serving is consumed. Placebo sachets will contain lactose, the carrier used in the active product. The total duration of the family's participation in the study will be approximately 4 weeks.

3.1 Study Endpoints

3.1.1. Primary Endpoint

Changes to the gut microbiome composition (including *B. infantis* colonization) in infants with FPIAP over a 4-week period (*B. infantis* INF108F vs placebo)

3.1.2. Secondary Endpoints

- The percentage of patients testing negative for blood in stool by Study Day 7, 14 and 28 by hemoccult testing
- The percentage of patients with no gross/visible blood in stool by Study Day 7, 14 and 28 by parental report
- Changes to stool frequency by Study Day 7, 14 and 28
- Changes to clinical symptoms including GER, feeding difficulties, sleep disturbance and poor growth
- The occurrence of supplement-related adverse events

3.1.3 Biomarkers / Exploratory Endpoints:

- Fecal calprotectin levels
- Fecal pH levels
- Fecal metabolomics
- Markers of inflammation and enteric cytokines including, but not limited to, IL-1, IL-8 and TNF-alpha in stool
- Levels of eosinophil-related granular proteins in stool
- Intestinal permeability/integrity markers including, but not limited to, lipocalin (NGAL)

3.2 Study Completion

This study will be considered "completed" when the primary and secondary objectives have been met. This includes the analysis of all the data required to meet the chosen objectives.

After the study is completed, the Principal Investigator and the Data Center will compile a final study report as per ICH E6 and 21CFR312. The study report will be submitted to the local IRB.

4. SUBJECT SELECTION

4.1 Inclusion Criteria

Individuals who meet all of the following criteria are eligible for enrollment as study participants. Potential candidates will be identified at the Pediatrics at Newton Wellesley (PNW) and MGH Pediatric Gastroenterology Clinic. The recruitment will occur at Newton Wellesley Hospital or Massachusetts General Hospital.

- Infants, male or female, of all ethnic/racial groups, with a gestational period of 37 to 42 weeks
- Infants aged 1-90 days old with a documented FPIAP with either gross blood or microscopic blood in stools, without other possible causes
- Infants must be exclusively breastfed or at least half of oral intake is from breast feeding or from expressed breast milk
- A willing parent or legal guardian will sign the consent form either electronically or with a wet ink signature

4.2 Exclusion Criteria

Individuals who meet any of these criteria are not eligible for enrollment as study participants:

- Infants born earlier than 37 weeks of gestation
- Infants who are exclusively formula-fed or less than half of oral intake is from breastfeeding or from expressed breast milk at the time of enrollment
- Infants born with medical complications (i.e., neurological, cerebral palsy, confirmed food allergies)
- Diagnosis of other severe or complicating medical problems, including autoimmune or chronic immune inflammatory conditions, gastrointestinal inflammatory conditions, or renal insufficiency
- History of abdominal surgery or congenital abnormalities of the GI track, the cardiovascular system, the pulmonary system or the renal system
- Antibiotic use (oral or systemic) within 7 days prior to enrollment
- Parents' intent to feed non-study probiotics or solid food to their infant at any time during the study
- Mothers with substance use disorder (SUD) or on Nicotine replacement therapy (NRT)
- Infants who have consumed any *B. infantis*-containing probiotics since birth

- Primary Immune Deficiency
- Maternal use of probiotics containing *B. infantis* during pregnancy, after the baby's birth and/or intent to use probiotics containing *B. infantis* at any time throughout the study
- Past or current medical problems or findings from physical examination or laboratory testing that are not listed above, which, in the opinion of the investigator, may pose additional risks from participation in the study, may interfere with the participant's ability to comply with study requirements, or that may impact the quality or interpretation of the data obtained from the study.

4.3 Participant Withdrawal Criteria

Participants may be terminated early from the study for the following reasons:

- The participant elects to withdraw consent from all future study activities, including follow-up.
- The participant is "lost to follow-up" (i.e., no further follow-up is possible because attempts to reestablish contact with the participant have failed).
- The participant dies.
- The participant develops a medical condition or is started on new medication(s) not previously mentioned in the list of prohibited medications that, in the opinion of the investigator, may pose additional risks from participation in the study, may interfere with the participant's ability to comply with study requirements or that may impact the quality of the data obtained from the study.
- The participant meets any of the individual stopping rules as delineated in Section 10.1.1.

Participants with early termination from this study will not be replaced.

5. SUBJECT ENROLLMENT

5.1 Recruitment and Pre-Screening

Breastfed infants with FPIAP, with either grossly visible bloody stool or microscopic bloody stools, excluding other causes, will be recruited at PNW or MGH Pediatric GI clinic. These individuals will be provided basic information about the study and ask potential participants to provide basic contact information and answer some study-specific questions as a pre-qualifier. Individuals who are interested to learn more will receive more details about the study, will be presented with an informed consent (IC), and will receive instructions as to how they are able to communicate with the study PI and other study team members to address any questions or concerns during the IC process and study duration.

5.2. Informed Consent Process

The parent of each infant participant will be required to sign an IRB-approved IC. Only participants for whom there is a signed and approved IC will be enrolled in the study. The IC will explain the study, and fully disclose its known risks and benefits by using simple, nontechnical terms wherever possible. The IC will include the elements required by the FDA in 21 CFR 50 and International Conference on Harmonization (ICH) guidelines. Parents will also be required to consent to the use of their protected health information (PHI) according to all applicable health information privacy regulations in the US. This authorization can be part of the IC or can be a separate document.

The investigator of the study is responsible for ensuring that a signed IC and authorization for use of PHI is collected from each participant that they enroll into the study at the time of that participant's enrollment. No study-related activities may be performed with, or by, any participant, and no data may be collected from any participant for study purposes prior to obtaining these consents and authorizations.

Participation in this study is voluntary and parents will be allowed to withdraw consent for their infant at any time for any reason with no impact on their eligibility to be treated for any adverse events or on their eligibility to receive other therapy.

5.3. Screening/Baseline

After signing the IC, parents will complete a screening questionnaire, demographics, medical history, and ConMeds to assess alignment with the eligibility criteria. Once eligibility at this point is confirmed, the study site will provide stool collection kits and the Study Product feeding kit. Parents will be provided with a binder, including logs and questionnaires that they will be expected to complete throughout the study.

Prior to start oral administration of *B. infantis* INF108F or placebo, the participant will have a physical exam with vital signs and parents will be expected to collect a baseline stool sample.

5.4. Randomization and Study Supply Shipment

Randomization lists will be maintained in a secured area by the study team. If the randomization key is shared such that blinding is compromised, the Principal Investigator will be notified.

Randomization will occur immediately after all screening and baseline tasks have been confirmed to be completed and baseline data has been deemed acceptable. Baseline tasks include:

- Confirming infant/mother eligibility
- Obtaining informed consent
- Confirming baseline data collection
- Confirming baseline stool specimen collection.

Only infants for whom acceptable baseline data and stool samples have been collected will be randomized into the supplementation phase of the study. Using the randomization key generated by an unblinded statistician and held by an unblinded study site team member, eligible infants will be assigned a randomization code and randomized to a study product arm. The unblinded team member will provide the enrolled patient's parents with

the appropriate Study Product Kit in accordance with the randomization key. Infants will be randomized in a 1:1 ratio to the following arms:

- 8.0 x 10^9 CFU *B. infantis* INF108F
- Lactose placebo

After randomization, the study team will provide the assigned feeding kit to the participant. Study supplies that will be provided include:

- Feeding kits to include 14 sachets of study product (*B. infantis* INF108F or lactose placebo depending on randomization), oral syringes, and mixing cups
- Stool collection kits

6. STUDY PROCEDURES

6.1 INVESTIGATIONAL PRODUCT(S)/INTERVENTION MATERIAL(S), OTHER STUDY PRODUCTS (CONTROLS/PLACEBOS)

6.1.1. Investigational Product(s) / Intervention(s)

INF108F

INF108F is formulated as water-soluble powder. The investigational study product is a probiotic, *B. infantis* INF108F. *B. longum* subsp. *infantis* INF108F (Infinant Health) was selected for this study due to its demonstrated ability to utilize many types of oligosaccharides found in breast milk (i.e., HMOs), including lacto-N-tetraose, lacto-N-neo-tetraose, 2'-fucosyllactose, 3'-fucosyllactose, 3'-sialyllactose, and 6'-sialyllactose.

The *B. infantis* INF108F was isolated from a healthy term breastfed infant. Its identity was confirmed by genetic sequencing of the organism by Infinant Health. Master stocks and working stocks were created and the strain is routinely tested for purity after fermentation. According to cGMP, the strain was re-isolated before creating the inoculum for the fermentation run.

The INF108F strain of *B. infantis* was produced by fermentation under cGMP conditions at a contract manufacturing organization (CMO). Following fermentation, the strain was centrifuged, freeze-dried, milled, and blended with lactose sourced from a Safe Quality Food (SQF) level 3 facility. The product was packaged by a second CMO into FDA compliant single-serving nitrogen-flushed polyester-faced laminated sachets with an oxygen and moisture barrier following cGMP. Each sachet contains approximately 600 mg of the blended powder containing at least 8 x 10^9 CFU *B. infantis* INF108F.

By way of its unique combination with human breast milk and utilization of HMOs, *B. infantis* INF108F is designated as a "Foods for Special Dietary Use" (FSDU). The term "special dietary uses", as applied to food for man, is described in Chapter 1, Subchapter B, Part 105 of the Code of Federal Regulations, 21 CFR and defined in Part 105.3. It means foods supplying particular dietary needs which exist by reason of a physical, physiological,

pathological or other condition, such as conditions of diseases, convalescence, pregnancy, lactation, allergic hypersensitivity to food, underweight and overweight. Included in the definition are uses for supplying particular dietary needs which exist by reason of age, including but not limited to the ages of infancy (not more than 12 months old) and childhood (more than 12 months but less than 12 years).

Within the FDA, the Office of Food Additive Safety oversees a process for evaluating the safety of food ingredients that have a history of use, and can, by scientific consensus, be generally regarded as safe (GRAS). These food ingredients must complete a rigorous process of scientific and data review by independent qualified experts, who assess whether the product is safe under the conditions of its intended use. *B. infantis* INF108F gained GRAS status after successfully completing this process, with unanimous consensus by all independent qualified experts that this product is generally recognized as safe based upon the application of generally available and accepted scientific data, information, or methods (i.e., peer-reviewed scientific literature), and scientific principles for use in term infants.

GRAS status for *B. infantis* INF108F was obtained for a use level of up to 2.8 x 10^{10} colony forming units (CFU) per day. The current commercial serving size of *B. infantis* INF108F is 8.0 x 10^{9} CFU/day.

INF108F Placebo:

INF108F Placebo is lactose® water soluble powder

The study product control is a lactose placebo. The lactose ingredient was sourced from a SQF level 3 facility and packaged into FDA compliant single-serving nitrogen-flushed polyester-faced laminated sachets with an oxygen and moisture barrier following cGMP. Each sachet contains 600 mg of lactose powder.

6.1.2 Study Product Handling, Storage and Compliance

Once a participant has been randomized, a feeding kit containing 14 sachets of *B. infantis* INF108F or placebo, oral syringes, and mixing cups will be provided. Parents will be instructed to store the supplement immediately upon receipt in their freezer until use.

Parents will be expected to record missed study product feedings in their daily feeding log. Compliance will be evaluated based on these data. Parents may also be requested at the end of the supplementation period to count the number of sachets remaining. Unused sachets may be kept by participants or disposed of.

6.1.3 Preparation and Administration

Parents will be trained on how to prepare and feed the study product to their infants. Infants enrolled into the control cohort will receive a lactose placebo and infants enrolled into the active cohort will receive *B. infantis* INF108F in lactose carrier. Both placebo and *B. infantis* INF108F feedings will be provided in powder form to be mixed in 3-5 mL (approximately 1 teaspoon) of breast milk each day for 14 consecutive days.

Study product cannot be initiated until 1) an infant has been deemed eligible for this study by the Investigator, 2) informed consent has been obtained, 3) the baseline stool sample has been collected and frozen by the parents, and 4) baseline study data has been obtained.

6.1.4 Accountability of Investigational Product(s) / Intervention(s)

Under Title 21 of the Code of Federal Regulations (21CFR §312.62) the investigator will maintain adequate records of the disposition of the investigational product(s) / intervention material(s), including the date and quantity of the product received, to whom the product was dispensed (participant-by-participant accounting), and a detailed accounting of any investigational product(s) / intervention material(s) accidentally or deliberately destroyed.

Records for receipt, storage, use, and disposition will be maintained by the study site. A Master Dispensation Log will be kept current. This log will contain the identification of each participant and the date and quantity of investigational product(s) / intervention material(s) dispensed. Any product that remains unused at the end of study will be destroyed.

All records regarding the disposition of the investigational product(s) / intervention material(s) will be available for inspection by the Clinical Research Associate (CRA).

6.1.5 Assessment of Compliance with Investigational Product(s) / Intervention Material(s)

Every study subject will be asked to record consumption of their feedings of INF108F/placebo in a daily feeding log. Investigational product compliance will be recorded as a percentage, calculated as the number of days of reported consumption divided by the number of days of supplementation expected.

For participants who are withdrawn prior to the end of the supplementation period, percent adherence will be based on the number of days active in the study prior to withdrawal.

6.1.6 Modification or Discontinuation of Investigational Product(s) / Intervention Material(s)

The parents may withdraw their infant from the study at any time by notifying the study team. Participants may be withdrawn from the study by the investigator if study procedures have not been followed or if the investigator determines the participant is not able to safely continue with the study due to an adverse event or other health-related issue. If greater or equal to 20 percent drop-out occurs, infants may be replaced to assure adequate statistical power.

6.2. OTHER MEDICATIONS

6.2.1 Concomitant Medications

Concomitant medications may be used as necessary throughout the study unless noted in section 6.2.

6.2.2 Prohibited Medications and Dietary Ingredients

Medications and dietary ingredients prohibited during the study include:

For Infant:

- Any probiotics (including infant formulas containing probiotics)
- Antibiotics (oral or systemic)
- Infant formula greater than half of the infant's daily nutrition intake
- Solid food

For Mother:

- Antibiotics
- Probiotics containing *B. infantis*

6.3. STUDY ASSESSMENTS AND PROCEDURES

6.3.1 Efficacy Assessments

6.3.1.1 Primary objective

The primary objective of the study is to assess changes to the gut microbiome composition (including *B. infantis* colonization) in infants with FPIAP over a 4-week period (B. infantis INF108F vs placebo)

6.3.1.2 Secondary Objectives

The secondary objectives of this study are to evaluate: clinical symptoms including blood in stool, stool frequency, GER, feeding difficulties, sleep disturbance and poor growth

6.3.1.2.1 Blood in stool Blood in stool will be assessed by daily hemoccult stool test on Days 0, 1-14, 21 (+/- 1 day), 28 (+/- 1 day)

6.3.1.2.2 Stooling Infant stooling will be assessed by daily log capturing frequency, consistency, night stooling, and presence of mucus or gross blood

6.3.1.2.3 Infant GER Infant GER will be assessed by standard Reflux Questionnaire on Day 7, 14, 21, and 28

6.3.1.2.4 Infant Sleep Infant sleep duration/quality will be assessed by Questionnaire on Day 7, 14, 21, and 28

6.3.1.2.5 Infant Feeding Infant feeding difficulties will be assessed by Questionnaire on Day 7, 14, 21, and 28

6.3.1.2.6 Infant Growth Infant growth will be monitored at weekly visits by measuring weight, length, and head circumference

6.3.1.2.7 Adverse Events Supplement-related adverse events will be recorded by parents in a health log, in consultation with the study team and PI.

6.3.2 Safety and Other Exploratory Assessments

Safety assessments will include parent-reported adverse events as described in Section 10.2.

6.3.2.1 Stool Sample Collection

In addition to being used for hemoccult testing, infant stool specimens will be analyzed as an exploratory objective of the study and will be collected once during the Baseline period and daily (when available) for Days 1-14, 21, and 28, and assessments may include the following markers:

- Fecal calprotectin levels
- Fecal pH levels
- Fecal metabolomics
- Markers of inflammation and enteric cytokines including, but not limited to, IL-1, IL-8 and TNF-alpha
- Levels of eosinophil-related granular proteins
- Intestinal permeability/integrity markers including, but not limited to, lipocalin (NGAL)

6.3.3 Stool Sample Documentation, Training, Storage, and Handling

6.3.3.1 Baseline Samples

Baseline stool swabs and chunks of stool must be collected before the first study supplementation is consumed. After collection, the parents will store the swab tubes (white top tube) at room temperature and will store the chunk tubes (brown top tube) in their home freezer in a bag provided to retain those samples. At the Day 7 in-person Study Visit, parents will bring all Days 0-7 stool samples (swab, chunk, and card) with them to the visit. At the end of the study, parents will bring all remaining stool samples to the study site at the Day 28 End of Study in-person Visit. All efforts should be made to collect stool samples within the target collection windows. Parents will be trained on how to collect the samples at enrollment and as needed throughout the study.

6.3.3.2 Stool Swabs

Starting with a freshly soiled diaper, the parent will first collect the stool swab sample using the supplies provided in the stool collection kit (green-topped tube containing a buffer/bead mixture and packaged swab). Parents will swab their infant's soiled diaper, collecting enough sample to entirely coat the head of the swab. They will place the stool-covered swab into a white-topped collection tube containing stabilization buffer and will snap the swab head off at the breakpoint, leaving the swab head in the tube. They will seal the tube and shake vigorously to ensure distribution of the stool within the stabilization buffer. Swab-containing tubes (white cap tube) will be stored at room temperature. .

6.3.3.3 Stool Chunks

Using the supplies provided in the stool collection kit (empty snap-cap tubes and white stool collection spatulas), mothers will collect as much loose stool sample from their infant's soiled diaper as possible before snapping the lid tightly closed for storage. They will likely need to take multiple scoops to fill the tube as much as possible. If little stool is available for collection in a particular diaper, mothers will be instructed to use subsequent soiled diapers that fall within the same stool collection window to add to the already frozen snap-cap tube.

For example: A mother can only collect the swab tube and a very small spatula-full of stool for the stool 'chunk' sample. If baby stools later within the same stool collection window, the mother will remove the previously frozen partial 'chunk' sample tube(s) from the freezer, add as much stool as possible and re-freeze the entire sample. She will not need to prepare a second swab sample and should not try to add more material to the green-topped tube.

6.3.3.4 Stool Sample Storage and Transport

Immediately after collecting their infant's stool swab and chunk samples, parents will store the whole chunk sample (tube with brown cap) in their home freezer within the study-provided freezer bag. Parents will bring the freezer bag containing the collected stool chunk samples on a provided frozen ice pack to the in-person Study Visit on Day 7 and the in-person Study Visit on Day 28.

Stool swab samples (tubes with white cap) should be stored at room temperature. Parents will bring the collected stool swab samples with them to the in-person Study Visit on Day 7 and the in-person Study Visit on Day 28.

Hemoccult cards should be stored at room temperature. Parents will bring the completed hemoccult cards with them to the Study Visits on day 7 and day 28.

6.3.3.5 Stool Sample Analysis

DNA and RNA will be extracted from stool swab samples and will be used for shotgun sequencing, next generation sequencing, and/or quantitative PCR to determine levels of *B. infantis*, total Bifidobacterium, microbial signatures, and relative abundance of the most abundant bacterial taxa. Biochemical analyses may be performed on stool chunk samples to evaluate markers as described in Section 6.3.2.

6.3.3.6 Stool Sample Retention by the Sponsor

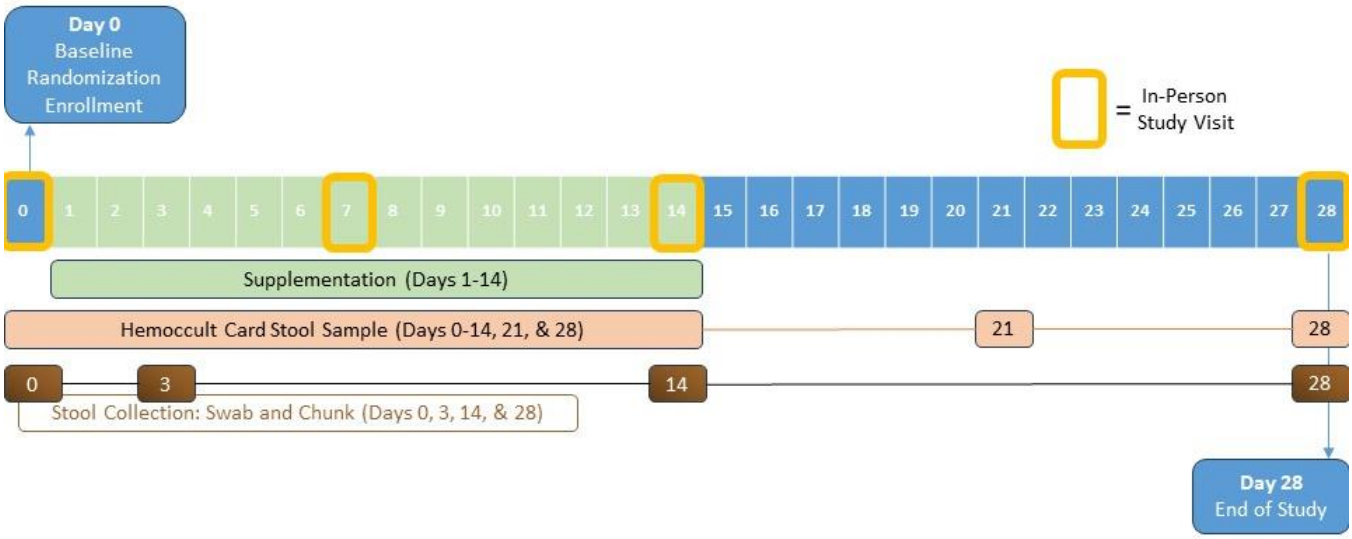
Infant stool samples collected during the conduct of the study may be kept by the Sponsor for up to 10 years for use in future research. No personally identifiable information will be recorded on stool specimen tubes.

6.4 Study Procedures

All study procedures are listed in **Table 1: Schedule of Events** below.

Table 1: Schedule of Events

	Screening	Day 0 (Baseline)	Days 1-6	Day 7	Days 8-13	Day 14	Days 15-27	Day 28 (End of Study)
Diagnosis	x							
Consent	x							
Screening	x							
Study Visit	x	x		x		x		x
Physical exam		x		x		x		x
Supplementation			x	x	x	x		
Questionnaire		x	x	x	x	x	x	
Feeding log		x	x	x	x	x	x	
Stooling log		x	x	x	x	x	x	
Clinician symptom tool		x		x		x		x
Randomization		x						
Stool collection		x	X Day 3			x		x
Hemoccult test		x	x	x	x	x	x	x
Stool to site				x				x
AE reporting			x	x	x	x	x	x



6.4.1 **Study Product Supplementation Period** Days 1-14

- Daily oral administration of *B. infantis* INF108F or placebo

- Daily stool collection
- Questionnaires for Sleep, Feeding and Stool frequency and consistency

On or after Day 28, study staff will check-in with parents to make sure that the final stool samples have been collected and all remaining Questionnaires have been completed. Parents will bring all remaining frozen stool samples to the Day 28 in-person study visit.

6.4.2 AE Follow-up

Days 15-28
Parents will report any post-supplementation period adverse events experienced by their infant during this time.

6.4.3 Compliance

Compliance will be determined by treatment consumption, as reported in the Daily Log. Treatment compliance will be recorded as a percentage, calculated as the number of days of reported consumption divided by the number of days of treatment expected. Missed assessments will be recorded. For participants who are withdrawn prior to the end of the treatment period, percent adherence will be based on the number of days active in the study prior to withdrawal.

6.4.4 Double-Blind Placebo-Controlled Administration of INF108F and Placebo

Prior to start of oral administration of *B. infantis* INF108F or placebo, the participant will have a physical exam with vital signs.

6.5 Participant Completion and Withdrawal

6.5.1 Participant Completion

Participants will have completed the study upon completion of Day 28 AE follow-up period.

6.5.2 Participant Withdrawal

The parents may withdraw their infant from the study at any time by notifying the study team. Participants may be withdrawn from the study by the investigator if study procedures have not been followed or if the investigator determines the participant is not able to safely continue with the study due to an adverse event or other health-related issue. If 20 percent drop-out occurs, infants may be replaced to ensure adequate statistical power.

6.6 Data Management

6.6.1 Data Collection

Study data will be collected on paper Case Report Forms and entered by study staff into a RedCap database.

6.6.2 Access to Data

The PI, study coordinators, and CRAs (Study Monitors) will only have access to the blinded study data.

6.6.3 Stool Collection

Stool samples will be collected from the participants at the following time points:

- At Screening / Baseline
- Day 3 (within a window of Study Day 3-5)
- Day 14 (+/- 1 day)
- Day 28 (+/- 1 day)

The stool samples will be banked and used for 16s rRNA or metagenomic sequencing for microbiome evaluation and biologic studies.

6.6.4 Physical Examination

This will include measurement of vital signs, including weight, temperature, heart rate, and blood pressure, and physical examination including the skin, ears, nose and throat, abdomen, lungs, and heart.

7. RISKS AND DISCOMFORTS

7.1 Risks of Investigational Product(s) / Intervention(s)

Previous studies have shown that *B. infantis* INF108F is safe and well-tolerated by infants (both term and preterm) with no known side effects.

7.2 Risk of Study Procedures

7.2.1 Double-Blind, Randomized, Placebo-Controlled clinical trial Possible adverse events during the clinical trial include mainly GI symptoms (gas, abdominal distension, vomiting, diarrhea, constipation or abdominal pain).

7.2.2 Administration Procedure Minimal risk is associated with administration of *B. infantis* INF108F, including choking, spit up, and vomiting.

7.2.3 Stool collection All stool collection will be from fresh diapers, which does not impose any potential risk to the infants.

8. BENEFITS

8.1. Benefits of Investigational Product(s) / Intervention(s)

The risks associated with participation in this study are minimal in relation to the potential benefits derived by both the subject and society. Participants may derive two major benefits from the study. First, participants may have faster resolution of FPIAP. Second, participants may have modified gut microbiota which could potentially lead to reduced risk, or even prevention of, other allergic diseases in life.

Potential benefits to society include a better understanding of modulation of the infant gut microbiome and the associated clinical outcomes in infants with FPIAP.

8.2 Benefits of Study Procedure(s)

With the treatment of *B infantis* INF108F, we anticipate that the infant's gut microbiome will likely be modified in a positive manner to correct the dysbiosis which leads to the development of FPIAP.

9. STATISTICAL ANALYSIS

9.1 SAMPLE SIZE CALCULATIONS AND STATISTICAL ANALYSIS PLAN

9.1.1 Determination of Sample Size

Approximately 100 infants and 100 mothers will be recruited and screened for the study to obtain a sample size of 50 participants for randomization, assuming a 50 percent screen fail rate. Forty (40) participants are expected to complete the study assuming a 20 percent drop-out rate. This sample size is based on a literature review of randomized clinical trials and the anticipated changes to the microbiome based on previous studies of *B. infantis*.

9.2 Data Analysis

9.2.1 General Considerations

Data will be collected and curated in a REDCap database [43] with support from the Human participants Core and exported into a suitable analysis format (e.g., STATA, SPSS, R analysis) for analysis. For continuous variables that

are normally distributed we will summarize them using mean with standard derivations and for those that are non-normally distributed, we will summarize with medians and inter-quartile ranges. For dichotomous data we will provide proportions. In addition to the baseline and outcome data, we will also summarize the recruitment numbers, those participants lost to follow-up, protocol violations and other relevant data.

9.2.2 Study Participant Populations

Randomization Approximately fifty (50) infants will be randomized in a 1:1 ratio to treatment probiotic or placebo. Randomization will occur immediately after all baseline period tasks have been confirmed to be completed and provide acceptable data.

Populations for Analyses The modified Intent to Treat (mITT) population will include all infants who are randomized and receive at least one supplementation. The Per Protocol (PP) population will include all randomized participants who do not have major protocol deviations. Major protocol deviations will be defined prior to unblinding of the data. Analysis on primary and secondary objectives will be completed based on the mITT population and the PP population; analysis on the exploratory objectives (perception of treatment) will be completed based on the mITT population.

Descriptive Statistics Descriptive statistics (mean, median, and standard deviation, minimum and maximum) of the quantitative variables will be summarized using number of subjects (n). The qualitative variables will be summarized using counts and percentages. All summaries will be presented by treatment group and time point (where relevant).

All CRF and laboratory data will be analyzed. Comparisons between treatment groups will be performed using Fisher's exact test or Pearson's Chi-Square test for categorical data and one-way ANOVA with Tukey's post hoc comparisons for individual timepoints or two-way repeated measures ANOVA for all timepoints for continuous data. Mean and standard deviations for certain groups and timepoints will be used as estimates in power calculations for future studies. Missing data will not be imputed in this study.

9.2.3 Efficacy Analysis

Primary Endpoint(s) The primary objective of the study is to assess the effectiveness of the probiotic, *B. infantis* INF108F, on modulating the gut microbiome composition of breastfed infants diagnosed with FPIAP from Baseline to Day 28, including colonization of INF108F. This will be measured using stool 16S analysis and quantitative PCR. Treatments will be compared using a Wilcoxon Rank Sum test.

Secondary Endpoint(s) The secondary objectives of this study as measured:

- Evaluating the reduction in stool frequency and improvement of stool consistency by Day 7 using Questionnaire
- Evaluating the improvement of infant GER symptoms by Day 14 using Questionnaire. Reduction by 50 percent from Baseline to Day 14 Infants with a reduction in the number of crying/fussing episodes of greater than or equal to 50 percent on Day 14 will be counted as a success. The number of successes per treatment group will be compared using a Chi-square or Fisher's Exact test, as appropriate.
- Evaluating the improvement of feeding difficulties by Day 14, using Questionnaire. The number of successes per treatment group will be compared using a Chi-square or Fisher's Exact test, as appropriate.

- Evaluating the improvement of infant's sleep by Day 14, using Questionnaire.
- Changes of stool cytokine profile by Day 28. The changes from baseline to Day 28 will be compared for the two groups using a repeated measures ANOVA.

Safety Analysis Fisher's Exact Test will also be used to compare treatment groups for SAEs and AEs resulting in withholding or discontinuing the study products. Adverse events will be summarized. Frequencies and percentages of subjects with treatment-emergent adverse events (TEAEs), serious TEAEs and TEAEs causing premature discontinuation will be provided by treatment group. An AE is treatment emergent if it 1) occurs after the first dose of randomized study treatment or 2) if it is present prior to receipt of randomized study treatment but worsens in severity or increases in frequency after the first dose of randomized study treatment.

9.2.4 Study Participant Baseline Characteristics and Demographics

Summary of descriptive statistics for baseline and demographic characteristics will be provided for all enrolled participants. Demographic data will include age, race, sex, body weight, and height; these data will be presented in summary tables.

9.2.5 Study Completion

Premature Termination of Study: Should it prove necessary to discontinue the study permanently prior to completion, the Sponsor will notify Infinant Health and the IRB of the rationale. Participants will be informed by the study site. All relevant study documents and data will then be returned to the Sponsor, and the study product will be destroyed or returned.

Termination of Study: After the completion or termination of the study, all relevant study documents and data will then be sent to the Sponsor and the study product will be destroyed. The Investigator will inform the IRB of the end of the study and a certificate of study closure will be issued.

9.3 Interim Analyses

One Interim Analysis will occur when the first 10 subjects (at least) complete the study. Interim Analysis data will be analyzed and reported by an unblinded statistician.

9.4 Deviations from Statistical Plan

The principal features of the study design and of the plan for statistical analysis of the data are outlined in this protocol. Any changes in these principal features will require a protocol amendment and will be described in the final report. Any such changes will be subject to review by the IRB.

9.5 Documenting an Unblinding

Any unblinding will require a full written account of the event(s) that necessitated the unblinding of the treatment assignment for an individual participant(s). This account will be made in the participant(s) study file and in the final study report and will include the reason(s) for the unblinding, the name of the Independent Study Monitor who was notified and approved the unblinding, the names of the individuals unblinded, and the date and time the unblinding occurred.

10 MONITORING AND QUALITY ASSURANCE

10.1 SAFETY PROCEDURES

10.1.1 Stopping Rules

10.1.1.1 Study Stopping Rules

Study enrollment and study procedures will be suspended pending expedited review of all pertinent data by the Partners institutional review board (IRB), the ISM, and the Infinant Medical Officer, if a participant at any time or in any group develops a severe or life-threatening adverse event such that he or she requires hospitalization, or an unexpected (non-allergy-related) hospitalization or death.

Study enrollment will be suspended pending expedited review of all pertinent data by the ISM if any of the following occur:

- Any death related to *B. infantis* INF108F
- Any serious adverse reaction to *B. infantis* INF108F

All above events will be reported immediately to the Partners IRB, the ISM, and Infinant's Medical Officer. The study will not resume until approval is given by the IRB, the ISM, and Infinant's Medical Officer.

10.1.1.2 Individual Stopping Rules

Safety of the participant will remain of primary importance. Any participant who develops documented hypotension (systolic BP>30% fall from baseline systolic BP), neurological compromise (confusion, loss of consciousness), and/or significant hypotension during any stage of the during any administration of the *B. infantis* product will be withdrawn from the study.

10.1.2 Early Discontinuation of Investigational Product(s) / Intervention(s) with continued study participation / follow-up

10.1.2.1 Unscheduled Termination In accordance with the Declaration of Helsinki, patients have the right to withdraw from the study at any time for any reason. Participants may withdraw with or without medical advice. The investigator also has the right to withdraw participants from the study. Participants will be removed from the

study for the following reasons: adverse experience, intercurrent illness or medication that in the judgment of the investigator may place the participant at risk, request of the investigator or participant for administrative or other reasons, protocol violation, determination that the participant is non-compliant or has unreliable behavior. Withdrawal from this study will have no impact on the future care of the participant at the Massachusetts General Hospital or any of its affiliated hospitals or health clinics.

10.1.3 Follow-up after early study termination

Participants who are prematurely terminated from the study will be followed to monitor safety for a minimum of 30 days or until resolution of the disqualifying event whichever is longer or until the Independent Study Monitor and the Principal Investigator determine that the follow-up is complete.

10.1.4 Participant Replacement

Participants who are permanently discontinued from the study will not be replaced.

10.2 ADVERSE EVENTS

This section defines the types of adverse events and outlines the procedures for appropriately collecting, grading, recording, and reporting them. Information in this section complies with ICH Guideline E2A: Clinical Safety Data Management: Definitions and Standards for Expedited Reporting and ICH E6: Guideline for Good Clinical Practice, and applies the standards set forth in the National Cancer Institute (NCI), Common Terminology Criteria for Adverse Events version 4.0. These criteria have been reviewed by the study investigators and have been determined appropriate for this study population.

10.2.1 Definitions

10.2.1.1 Adverse Events An adverse event (AE) is any occurrence or worsening of an undesirable or unintended sign, symptom, laboratory finding, or disease that is experienced during participation in the trial. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporarily associated with the use of a medicinal (investigational) Study Agent(s) whether related to the medicinal (investigational) Study Agent(s) or not. Any medical condition that is present at the time that the participant is screened will be considered as baseline and not recorded as an AE. However, if the condition deteriorates or changes in severity at any time during the study it will be recorded and reported as an AE.

10.2.1.2 Suspected Adverse Reaction and Adverse Reaction Suspected adverse reaction (SAR) means any adverse event for which there is a reasonable possibility that the drug caused the adverse event. For the purposes of safety reporting, “reasonable possibility” means there is evidence to suggest a causal relationship between the drug and the adverse event.

An adverse reaction (AR) means any adverse event caused by a drug. Adverse reactions are a subset of all suspected adverse reactions for which there is reason to conclude that the drug caused the event. A suspected

adverse reaction implies a lesser degree of certainty about causality than adverse reaction, which means any adverse event caused by a drug.

10.2.1.3 Adverse Events Associated with Study Procedures The following clinical situations, when associated with study procedures, are defined as adverse events and will be recorded on the AE CRF. These situations do not limit the principal investigator from recording and reporting any other events as AEs, associated or not with these procedures.

10.2.1.4 Serious Adverse Event (SAE) An AE or SAR (including AR) is considered serious if, in the view of the investigator, it results in any of the following outcomes (21 CFR 312.32):

- Death. A death that occurs during the study or that comes to the attention of the investigator during the protocol-defined follow-up.

- A life-threatening event. A life-threatening event is any adverse experience that, in the view of the investigator, places the study participant at immediate risk of death from the reaction as it occurred. It does not include an adverse event or suspected adverse reaction that, had it occurred in a more severe form, might have caused death.

- An inpatient hospitalization or prolongation of existing hospitalization.

- Persistent or significant disability/incapacity or substantial disruption of the ability to conduct normal life functions.
- An important medical event that may not result in death, be life threatening, or require hospitalization may be considered an SAE when, based on appropriate medical judgment, it may jeopardize the participant and may require medical or surgical intervention to prevent one of the outcomes listed above.

- Congenital anomaly or birth defect.

Regardless of the relationship of the adverse event to the study, the event will be reported per Section 8.2.2.3 as an SAE if it meets any of the above definitions.

10.2.1.5 Unexpected Adverse Event An AE or SAR (including AR) is considered "unexpected" if it is not consistent with the risk information described in the study protocol.

10.2.2 Collecting, Recording and Managing Adverse Events

10.2.2.1 Identifying Adverse Events Any adverse event that occurs from the moment the participant has signed the consent form will be recorded and is reportable. Adverse events will be reported until the participant has completed the long-term follow-up phase.

Adverse events may be discovered through any of these methods:

- Observing the participant.
- Questioning the participant with standardized questions/procedures.
- Receiving an unsolicited complaint from the participant.
- An abnormal value or result from a clinical or laboratory evaluation (e.g., a radiograph, an ultrasound, or an electrocardiogram) can also indicate an adverse event.

Adverse events will be captured as follows: (1) every occurrence as per the NCI-CTCAE criteria, (2) for protocol-specific adverse events (Section 10.2.1.3) or (3) when determined to be clinically significant by the Principal Investigator.

All adverse events occurring during or within 24 hours of the study procedures will be reported as an adverse event.

10.2.2.2 Recording AEs Throughout the study, all identified adverse events (serious and non-serious) will be recorded on all appropriate source document and adverse event case report forms regardless of their severity or relation to the study.

A complete description of all adverse events will include event description, time of onset, investigator assessment of severity, relationship to study agent(s) or procedures/intervention(s), time of resolution/stabilization of the event, expectedness, determination of whether the AE qualifies as a SAE, and action taken. A change in the severity of the AE will also be documented. The PI will document assessment of severity and relationship on the source documents or the on the CRF.

10.2.2.3 Recording SAEs Serious adverse events will be recorded on the serious adverse event case report form and will include a narrative of the event signed and dated by the Principal Investigator and the Independent Safety Monitor.

10.2.2.4 Managing Adverse Events The site investigator must apply his or her clinical judgment as to whether an AE is of sufficient severity to require that the participant immediately be removed from further treatment under the protocol. The investigator must institute any necessary medical therapy to protect a participant from any immediate risk.

There is no risk for possible anaphylaxis with our study agent INF108F and placebo. Epinephrine auto injectors and diphenhydramine will not be needed. Potential gastrointestinal adverse effects might occur, including abdominal discomfort and changes of stool frequency and consistency. We will stop administering the study agent if any of adverse events occur and we will treat the symptoms accordingly.

The same protocol will be recommended when signs/symptoms of acute gastrointestinal infection occur. An adverse event will be followed until any of the following takes place: a) it is resolved, b) participant is stable, c) a minimum of 30 days after participant is terminated from the study and the Independent Safety Monitor and the

Principal Investigator determine that follow-up is complete. If an abnormal value or result is determined by the investigator to be clinically significant, it must be recorded as an adverse event on the appropriate laboratory evaluation form(s).

10.2.3 Grading and Attribution

10.2.3.1 Grading criteria In addition to determining whether an adverse event fulfills criteria for a serious adverse event or not, the severity of adverse events experienced by study participants will be graded according to the criteria set forth in the National Cancer Institute's Common Terminology Criteria for Adverse Events Version 4.0. This document (referred to herein as the NCI-CTCAE manual) provides a common language to describe levels of severity, to analyze and interpret data, and to articulate the clinical significance of all adverse events. The grading criteria listed below will supersede any in the NCI-CTCAE manual.

All adverse events whether or not listed in the NCI-CTCAE will be graded on a scale from 1 to 5 according to the following standards in the NCI-CTCAE manual (A semi-colon indicates "or" within the description of the grade.):

- Grade 1 = Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.

- Grade 2 = Moderate; minimal, local, or noninvasive intervention indicated; limiting age-appropriate instrumental ADL (preparing meals, shopping for groceries or clothes, using the telephone, money, etc.).

- Grade 3 = Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care ADL (bathing, dressing and undressing, feeding self, using he toilet, taking medications, and not bedridden).

- Grade 4 = Life-threatening consequences; or urgent intervention indicated.

- Grade 5 = Death related to AE.

Adverse events not included in the NCI-CTCAE listing or which have relative specificity for this protocol will be recorded and graded 1 to 5 according to the grade definition provided below:

ADVERSE EVENT: CARDIAC ARRHYTHMIA (CONDUCTION DISORDER)
Definition: A disorder characterized by pathological irregularities in the cardiac conduction system.
Grade 1 = Mild symptoms; intervention not indicated
Grade 2 = Moderate symptoms
Grade 3 = Severe symptoms; intervention indicated
Grade 4 = Life-threatening consequences; urgent intervention indicated
Grade 5 = Death

ADVERSE EVENT: BRONCHOSPASM
Definition: A disorder characterized by a sudden contraction of the smooth muscles of the bronchial wall.
Grade 1 = Mild symptoms; intervention not indicated
Grade 2 = Symptomatic; medical intervention indicated; limiting instrumental ADL
Grade 3 = Limiting self care ADL; oxygen saturation decreased
Grade 4 = Life-threatening respiratory or hemodynamic compromise; intubation or urgent intervention indicated Grade 5 = Death

ADVERSE EVENT: COUGH
Definition: A disorder characterized by sudden, often repetitive, spasmodic contraction of the thoracic cavity, resulting in violent release of air from the lungs and usually accompanied by a distinctive sound.
Grade 1 = Mild symptoms; nonprescription intervention indicated
Grade 2 = Moderate symptoms, medical intervention indicated; limiting instrumental ADL
Grade 3 = Severe symptoms; limiting self care ADL
Grade 4 = N/A
Grade 5 = N/A

ADVERSE EVENT: FEVER
Definition: A disorder characterized by elevation of the body's temperature above the upper limit of normal.
Grade 1= 38.0 – 39.0 degrees C (100.4- 102.2 degrees F) despite use of acetaminophen
Grade 2= >39.0 – 40.0 degrees C (102.3- 104.0 degrees F) despite use of acetaminophen Grade 3= >40.0 degrees C (>104.0degrees F) for <24 hrs despite use of acetaminophen
Grade 4= >40.0 degrees C (>104.0degrees F) for >24 hrs despite use of acetaminophen Grade 5= Death

ADVERSE EVENT: NAUSEA
Definition: A disorder characterized by a queasy sensation and/or the urge to vomit.
Grade 1 = Loss of appetite without alteration in eating habits
Grade 2 = Oral intake decreased without significant weight loss, dehydration or malnutrition
Grade 3 = Inadequate oral caloric or fluid intake, likely associated with losses due to vomiting; hospitalization may be warranted Grade 4 = N/A
Grade 5 = N/A

ADVERSE EVENT: ABDOMINAL PAIN
Definition: A disorder characterized by pain or discomfort localized to the abdominal cavity.
Grade 1 = Mild: possibly associated with loss of appetite without alteration in eating habits, or normal activity
Grade 2 = Moderate: Oral intake and normal activity decreased without significant weight loss, dehydration or malnutrition
Grade 3 = Severe: Inadequate oral caloric or fluid intake; debilitating pain causing significant curtailment of normal activity Grade 4 = N/A
Grade 5 = N/A

10.2.3.2 Definition of Attribution The attribution of an adverse event to the study will initially be determined by the Principal Investigator or designated physician co/sub-investigator. The Principal Investigator or designee will record the determination of attribution on the appropriate adverse event or serious adverse event form. The attribution of an adverse event to the investigational drug(s) or other study drug (s) will be determined using the descriptors in the following table.

For the purpose of this study, in addition to all study medications, the following interventions/procedures will be considered when determining attribution:

10.2.3.2.1 Products

- *B. infantis* INF108F powder
- Placebo INF108F powder

10.2.3.2.2 Interventions

- Double-Blind Placebo-Controlled administration of *B. infantis* INF108F or Placebo

Code	Descriptor	Definition (guidelines)

UNRELATED CATEGORIES

1	Unrelated	The adverse event is clearly not related to study. The event is completely related to an etiology other than the study product or study intervention (the alternative etiology must be documented in the study participant's medical record).
2	Unlikely	The adverse event is doubtfully related to study and likely to be related to factors other than study product or study intervention.

RELATED CATEGORIES

3	Possible	The adverse event may be related to study. There is an association between the event and the administration of study product and there is a plausible mechanism for the event to be related to the study product; there may be also an alternative etiology, such as characteristics of the participant's clinical status and/or underlying disease.
4	Probable	The adverse event is likely related to study. There is (1) an association between the event and the administration of study product or study intervention, (2) a plausible mechanism for the event to be related to the study product, and (3) the event could not be reasonably explained by known characteristics of the participant's clinical status and or an alternative etiology is not apparent.
5	Definite	The adverse event is clearly related to study. There is (1) an association between the event and the administration of the study product or study intervention, (2) a plausible mechanism for the event to be related to the related to the study product, and (3) causes other than the study product have been ruled out and/or the event re-appeared on re-exposure to the study product.

(For additional information and a printable version of the NCI-CTCAE manual, consult the NCI-CTCAE website: http://ctep.cancer.gov/reporting/ctc.html)

In a clinical trial, the study product/intervention will always be suspect when attributing an AE and the "unrelated" attribution will be used only when there is an indisputable or likely alternative explanation for the AE.

10.2.4 SAE Reporting Criteria and Procedures

The Principal Investigator will be notified by the study staff as soon as a staff member becomes aware of the SAE. In the absence of the Principal Investigator, a physician sub-investigator will be notified.

10.2.4.1 Notifying Infinant's Medical Officer Infinant's Medical Officer and the Independent Safety Monitor will be notified by the Principal Investigator no later than 24 hours after the investigative site becomes aware of the SAE, regardless of the presumed relationship to the study product. Reporting to Infinant's Medical Officer will utilize an initial SAE case report form in draft format. Contact information for Infinant's Medical Officer is listed below:

Karl Sylvester, MD
Infinant Health
2121 2nd Street
Davis, CA 95616
Tel 650-804-0597
ksylvester@infinanthealth.com

Within another 24 hours, Infinant's Medical Officer, Independent Safety Monitor, and the Principal Investigator will discuss the impact of the SAE on the participant and on the study and Infinant's Medical Officer will decide

whether standard or expedited reporting will be applied. A finalized, SAE case report form will be generated by the Principal Investigator and must be approved by Infinant's Medical Officer. The finalized, Infinant Health-approved case report form will be placed in the participant study chart and will be sent to the Independent Safety Monitor. As additional clinical information is obtained by the Principal Investigator regarding the SAE, the SAE case report will be revised and submitted to Infinant's Medical Officer and the Independent Safety Monitor.

10.2.4.2 Unexpected, Non-Serious Adverse Events An unexpected, non-serious adverse event that is of Grade 2 severity or higher and study-related will be recorded and reported to the Independent Safety Monitor and Infinant's Medical Officer under the serious adverse event reporting procedure outlined in the SAE Reporting and Criteria Section (Section 8) of the protocol (i.e., within 24 hours).

10.2.4.3 Notifying the Data and Independent Safety Monitor (ISM) The Principal Investigator is responsible for submitting all expedited SAEs on an ongoing basis to the Independent Safety Monitor. Individual or clusters of SAEs may be reported expeditiously to the ISM either when specified by the ISM, or upon determination of Infinant's Medical Officer.

10.2.4.4 Notifying the Institutional Review Board The Principal Investigator will ensure the timely dissemination of SAE information, including expedited reports, to the IRB in accordance with IRB regulations and guidelines.

10.2.5 Non-Serious Adverse Events (NSAES) Reporting

10.2.5.1 Notifying the Independent Safety Monitor The Principal Investigator will provide the Independent Safety Monitor with a listing of all AEs in a Vedanta Biosciences-provided standard format and timeline for review during planned protocol reviews. Individual or clusters of AEs may be reported expeditiously to the ISM either when specified by the ISM, or upon determination of Infinant's Medical Officer.

10.2.5.2 Notifying the Institutional Review Board The Principal Investigator will ensure the timely dissemination of AE information to the IRB in accordance with applicable regulations and guidelines.

10.3 PROTOCOL DEVIATIONS

The practices below are consistent with Good Clinical Practice (GCP ICH E6) Sections:

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

10.3.1 Protocol Deviation Definitions

10.3.1.1 Protocol Deviation – Any change, divergence, or departure from the study design or procedures of a research protocol that affects the participant's rights, safety, or well-being and/or the completeness, accuracy and reliability of the study data constitutes a protocol violation. Changes or alterations in the conduct of the trial which do not have a major impact on the participant's rights, safety or well-being, or the completeness, accuracy

and reliability of the study data are considered minor protocol deviations. The Principal Investigator is responsible for reporting protocol deviations to the IRB using the standard reporting form. As a result of deviations, corrective actions are to be developed by the site and implemented promptly.

10.3.1.2 Major Protocol Deviation – A protocol violation is a deviation from the IRB approved protocol that may affect the participant's rights, safety, or well-being and/or the completeness, accuracy, and reliability of the study data.

If the deviation meets any of the following criteria, it is considered a major protocol deviation (protocol violation). Example list is not exhaustive.

1. The deviation has harmed or posed a significant or substantive risk of harm to the research participant.
   Examples:
   - A research participant received the wrong treatment or incorrect dose.
   - A research participant met withdrawal criteria during the study but was not withdrawn.
2. The deviation compromises the scientific integrity of the data collected for the study. Examples:
   - A research participant was enrolled but does not meet the protocol's eligibility criteria.
   - Failure to treat research participants per protocol procedures that specifically relate to primary efficacy outcomes (if it involves patient safety it meets the first category above) – Changing the protocol without prior IRB approval.
   - Inadvertent loss of samples or data.
3. The deviation is a willful or knowing breach of human participant protection regulations, policies, or procedures on the part of the investigator(s). Examples:
   - Failure to obtain informed consent prior to initiation of study-related Procedures
   - Use of outdated or incorrect consent forms
   - Falsifying research or medical records
   - Performing tests or procedures beyond the individual's professional scope or privilege status (credentialing)
4. The deviation involves a serious or continuing noncompliance with federal, state, local or institutional human participant protection regulations, policies, or procedures. Examples:
   - Working under an expired professional license or certification
   - Failure to follow federal and/or local regulations, and intramural research – Repeated minor deviations.
5. The deviation is inconsistent with the NIH Human Research Protection Program's research, medical, and ethical principles. Examples:

- A breach of confidentiality.
- Inadequate or improper informed consent procedure.

10.3.1.3 Non-Major Protocol Deviation A non-major protocol deviation is any change, divergence, or departure from the study design or procedures of a research protocol that has not been approved by the IRB and which DOES NOT have a major impact on the participant's rights, safety or well-being, or the completeness, accuracy, and reliability of the study data.

10.3.2 Reporting Protocol Deviations

Upon determination that a protocol deviation has occurred, the study staff will a) notify the Principal Investigator, b) notify the Infinant Health Project Manager (refer to investigator's signature page for contact information) and c) will complete the Protocol Deviation form. Infinant Health may request discussion with the Principal Investigator and the Independent Safety Monitor to determine the effect of the protocol deviation on the study participant and his/her further study participation, the effect of the protocol deviation on the overall study and corrective actions. The Principal Investigator will complete and sign the Protocol Deviation form and submit it to Infinant's Medical Officer and Project Manager, to the Independent Safety Monitor and to the site IRB, per IRB regulations. Major protocol deviations will be reported to the ISM and Infinant's Medical Officer.

All study staff be educated about the adverse event reporting policy and will be instructed to notify an investigator if an event occurs.

10.4 QUALITY CONTROL AND QUALITY ASSURANCE

The Principal Investigator will keep accurate records to ensure that the conduct of the study is fully documented. The investigator will ensure that all CRFs and participant study files are legible and complete for every participant.

The Principal Investigator, through the use of a Clinical Research Associate (CRA), will be responsible for the regular review of the conduct of the trial, for verifying adherence to the protocol, and for confirming the completeness, consistency, and accuracy of all documented data and accuracy of source documentation verification. The reports of the CRA will be submitted to the Principal Investigator and the Infinant Health Project Manager. Infinant Health will independently review these reports.

When the CRFs are complete, they will be reviewed and signed by the Principal Investigator. All discrepancies identified will be reviewed, and any resulting queries will be resolved with the Principal Investigator and the CRFs will be amended as needed.

10.5 ETHICAL CONSIDERATIONS AND COMPLIANCE WITH GOOD CLINICAL PRACTICE

10.5.1 Statement of Compliance

This study was designed to ensure the protection of participants according to the ethical principles of the Declaration of Helsinki and amendments concerning medical research in human participants. This clinical study will be conducted using current good clinical practice (cGCP), as delineated in Guidance for Industry: E6 Good Clinical Practice Consolidated Guidance 1, and according to the criteria specified in this study protocol. Before study initiation, the protocol and the informed consent documents will be reviewed and approved by the local IRB. Any amendments to the protocol or to the consent materials will also be approved by the local IRB prior to implementation.

10.6 Informed Consent

The informed consent form will provide information about the study to a prospective participant or participant's legal representative to allow for an informed decision about participation in the study. All participants (or their legally acceptable representative) must read, sign (electronically or with wet ink), and date a consent form prior to study participation. The electronic consent process will be FDA 21 CFR Part 11 compliant.

The informed consent form will be revised and receive IRB approval whenever important new safety information is available, whenever the protocol is amended, and/or whenever any new information becomes available that may affect participation in the trial.

A copy of the appropriate informed consent form will be given to a prospective participant for review. The Principal Investigator or an approved designee, will discuss the consent with the prospective participant and answer questions, either in person or via institution-approved video conferencing platform. The prospective participant will be told that being in the trial is voluntary and that he or she may withdraw from the study at any time, for any reason.

11. Privacy and Confidentiality

A participant's privacy and confidentiality will be respected throughout the study. Each participant will be assigned a sequential identification number and these numbers rather than names will be used during collection, storage, and reporting of participant information.

- ☒ Study procedures will be conducted in a private setting
- ☒ Only data and/or specimens necessary for the conduct of the study will be collected
- ☒ Data collected (paper and/or electronic) will be maintained in a secure location with appropriate protections such as password protection, encryption, physical security measures (locked files/areas)

- ☒ Specimens collected will be maintained in a secure location with appropriate protections (e.g. locked storage spaces, laboratory areas)
- ☒ Data and specimens will only be shared with individuals who are members of the IRB-approved research team or approved for sharing as described in this IRB protocol
- ☒ Data and/or specimens requiring transportation from one location or electronic space to another will be transported only in a secure manner (e.g. encrypted files, password protection, using chain-of-custody procedures, etc.)
- ☒ All electronic communication with participants will comply with Mass General Brigham secure communication policies
- ☒ Identifiers will be coded or removed as soon as feasible and access to files linking identifiers with coded data or specimens will be limited to the minimal necessary members of the research team required to conduct the research
- ☒ All staff are trained on and will follow the Mass General Brigham policies and procedures for maintaining appropriate confidentiality of research data and specimens
- ☒ The PI will ensure that all staff implement and follow any Research Information Service Office (RISO) requirements for this research
- ☒ Additional privacy and/or confidentiality protections

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APPENDIX A

Data Monitoring Committee / Data and Safety Monitoring Board Appendix

There is no separate Data Monitoring Committee (DMC) or Data and Safety Monitoring Board (DSMB) for this study. We have specific procedures to ensure the quality and monitor safety for the study as described in Section 10.

- *To be completed for studies monitored by Data Monitoring Committee (DMC) or Data and Safety Monitoring Board (DSMB) if a full DMC/DSMB charter is not available at the time of initial IRB review.*
- *DMC/DSMB Charter and/or Roster can be submitted to the IRB later via Amendment, though these are not required.*

A Data Monitoring Committee (DMC) or Data and Safety Monitoring Board (DSMB) will be convened for safety monitoring of this research study. The following characteristics describe the DMC/DSMB convened for this study (Check all that apply):

☐ The DMC/DSMB is independent from the study team and study sponsor.

☐ A process has been implemented to ensure absence of conflicts of interest by DMC/DSMB members.

☐ The DMC/DSMB has the authority to intervene on study progress in the event of safety concerns, e.g., to suspend or terminate a study if new safety concerns have been identified or need to be investigated.

☐ Describe number and types of (i.e., qualifications of) members:
Click or tap here to enter text.

☐ Describe planned frequency of meetings:
Click or tap here to enter text.

☐ DMC/DSMB reports with no findings (i.e., “continue without modifications”) will be submitted to the IRB at the time of Continuing Review.

☐ DMC/DSMB reports with findings/modifications required will be submitted promptly (within 5 business days/7 calendar days of becoming aware) to the IRB as an Other Event.