Title Page

Protocol title:

A Phase 3, randomized, double-blind, placebo-controlled study to evaluate the effect of Bi-26 (strain of *Bifidobacterium longum, B. infantis)* supplementation versus placebo on weight gain in underweight infants

Protocol Number: Gates MRI-MNK01-301

Version: 4.0

Compound: *Bifidobacterium longum* subspecies *infantis* (*B. infantis*) strain Bi-26™, product code MNK01

Study Phase: Phase 3

Short Title: Impact of Bi-26 supplementation on weight gain in underweight infants

Sponsor Name: Bill & Melinda Gates Medical Research Institute

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Medical Monitor and other important study personnel information will be provided in a separate document.

Approval Date: 12 Sept 2022

Sponsor Signature Page

See appended electronic signature page

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Sponsor Signatory Date

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Biostatistician Date

[redacted], DrPH

Head of Biometrics & Data Management

Bill & Melinda Gates Medical Research Institute

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Chief Medical Officer

Bill & Melinda Gates Medical Research Institute

Principal Investigator Signature Page

I have read the protocol, appendices, and accessory materials related to Gates MRI-MNK01-301 entitled "A Phase 3, randomized, double-blind, placebo-controlled study to evaluate the effect of Bi-26 (strain of *Bifidobacterium longum, B. infantis*) supplementation versus placebo on weight gain in underweight infants", and agree to the following:

- To conduct this study as described by the protocol and any accessory materials
- To protect the rights, safety, and welfare of the participants under my care
- To provide oversight to all personnel to whom study activities have been delegated
- To control all investigational products provided by the sponsor and maintain records of the disposition of those products
- To conduct the study in accordance with:
    - consensus ethical principles derived from international guidelines including the Declaration of Helsinki and Council for International Organizations of Medical Sciences (CIOMS) International Ethical Guidelines
    - current International Council for Harmonization Guideline for Good Clinical Practice (GCP)
    - applicable laws and regulations
- To obtain approval for the protocol and all written materials provided to participants prior to initiating the study at my site
- To obtain informed consent – and updated consent in the event of new information or amendments – from all participants enrolled at my study site prior to initiating any study-specific procedures or administering investigational products to those participants
- To maintain study records of each participant and all data required by the protocol.

_____ _____

Principal Investigator Signatory Date

Name:

Title:

Affiliation:

City and Country:

Protocol Version History and Summary of Changes

DOCUMENT HISTORY

Document	Date
Version 4.0	12 Sept 2022
Version 3.0	15 Aug 2022
Version 2.0	27 June 2022
Original Protocol Version 1.0	28 April 2022

Overall Rationale for changes in Version 4.0:

The purpose of this substantial protocol amendment was to add an exclusion criterion as described in the table below.

Section # and Name	Description of Change	Brief Rationale
Section 5.2 Exclusion Criteria	Added new criterion (8. Ongoing infant antibiotic (e.g., as prophylaxis in sickle cell disease) and/or probiotic usage), and updated subsequent criteria numbering	Included to prevent confounding of study results.

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List of Abbreviations

AE	Adverse event
CFU	Colony forming units
CRF	Case Report Form, Electronic Case Report Form
CRO	Contract research organization
EDC	Electronic data capture
EoS	End of study
GCP	Good Clinical Practice
GRAS	Generally Recognized as Safe
HMO	Human milk oligosaccharides
ICF	Informed consent form
ICH	International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use
IDMC	Independent Data Monitoring Committee
IRB	Institutional Review Board (ethics committee)
ITT	Intention-to-treat
IWRS	Interactive Web Response System
LMIC	Low- and middle-income countries
mITT	modified intention-to-treat
MUAC	Mid-upper arm circumference
NEC	Necrotizing enterocolitis
PP	Per-protocol
SAE	Serious adverse event
SAM	Severe acute malnutrition
SAP	Statistical analysis plan
SoA	Schedule of activities
US	United States
WAZ	Weight-for-age Z score
WHO	World Health Organization
WLZ	Weight-for-length Z score

1. Protocol Summary

1.1. Synopsis

Protocol Title: A Phase 3, randomized, double-blind, placebo-controlled study to evaluate the effect of Bi-26 (strain of *Bifidobacterium longum, B. infantis)* supplementation versus placebo on weight gain in underweight infants

Short Title: Impact of Bi-26 supplementation on weight gain in underweight infants

Rationale: Bi-26 is a nutritional probiotic intervention that is being evaluated for its impact on weight gain and other health outcomes in underweight infants who have been hospitalized with acute illness.

The burden of disease experienced by severely malnourished infants is significant in low-income countries. Gut dysbiosis, an imbalance in microbial composition, is thought to play a role in nutrient malabsorption leading to underweight infants and failure to thrive. *B. infantis* is thought to play an important role in the breakdown and adsorption of human milk oligosaccharides (HMOs) [Duar 2020; Casaburi 2021].

Supplementation with *B. infantis* strain EVC001 has been shown to colonize the gut and is associated with significant changes to fecal microbiome composition in healthy breast-fed infants [Frese 2017; Henrick 2019]. Supplementation of *B. infantis* has also been associated with a reduction in pro-inflammatory cytokines [Henrick 2019]. In a pilot trial evaluating *B. infantis* supplementation with strain EVC001 in hospitalized 2- to 6-month-old infants with severe acute malnutrition (SAM) in Bangladesh, daily supplementation of *B. infantis* for 4 weeks was associated with *B. infantis* colonization and an increase in weight-for-age Z score (WAZ) [Barratt 2022].

If confirmed in a larger clinical trial, *B. infantis* strains, such as Bi-26 used in this study, could potentially be used as a dietary supplement in underweight infants in low-income countries.

Overall Design

Disclosure statement: This is a Phase 3, randomized, double-blind, placebo-controlled study to demonstrate increased weight gain with Bi-26 supplementation versus placebo in underweight infants (WAZ<-2) discharged from hospital following acute illness and to evaluate the safety of Bi-26 supplementation in this population.

Underweight infants (WAZ<-2), who are between 1 and 4 months of age at enrollment and are currently hospitalized for acute non-surgical illness, will be enrolled to receive daily supplementation of Bi-26 or placebo for 28 days with a total study duration of approximately 90 days.

Intervention:

The Bi-26 supplement is presented as a lyophilized powder. A single dose of supplement will be resuspended and administered to the participant each day for 28 days. Once a day, the mother mixes the powder with approximately 3 mL to 5 mL of breastmilk and administers to the infant orally using a feeding syringe. In keeping with current World Health Organization (WHO) recommendation that children are exclusively breast-fed for the first 6 months of life [WHO 2022], breastmilk is preferred for mixing the supplement. If the mother is unable to express breastmilk, the powder may be mixed in approximately 3 mL to 5 mL of water.

While a total of 7 doses of Bi-26 supplement are to be administered per week, one each day, 9 doses will be provided each week to allow for an additional 2 doses, if needed, for repeat dose administration in the event of vomiting, or unexpected events which may render a dose unusable (e.g., spillage or otherwise compromised). Any additional dose/s not administered will be collected by study staff at the following visit.

Doses will either *1)* be delivered to the mother by study staff, or *2)* stored by staff, at the local health center to be picked up from the health center.

Study activities are assigned to the mother of the infant participant because the preferred method of reconstitution of the study intervention is in breastmilk. However, other caretakers may perform certain study activities (e.g., picking up the study doses, assisting in completion of the feeding diary, etc.).

Two treatment groups, shown below, will be enrolled in parallel.

Intervention	Duration of Study Intervention	Number of Participants Randomized
Bi-26	28 days	198
Placebo	28 days	198

Number of Participants: A sufficient number of participants will be screened to randomize approximately 396 study participants.

Screening (within a week before anticipated hospital discharge and enrollment/randomization): Informed consent will be obtained, and screening activities will take place during the infant's hospitalization. If a participant is discharged to a nutrition rehabilitation unit, this would still be considered a hospital discharge.

Enrollment and Randomization (day of hospital discharge): After the infant participant has completed the acute stabilization phase of treatment, including fluid rehydration and antibiotic course as needed, eligibility criteria will be checked and verified on study Day 1, the day of hospital discharge. Eligible participants will be enrolled and randomized at the time of hospital discharge.

Note: Screening may be conducted on the same day as enrollment/randomization (Day 1). Study assessments should not be duplicated if screening and Day 1 occur on the same day.

To minimize differences between groups, randomization will be stratified by:

- country
- age at enrollment (30-59 days of age, 60-120 days of age)
- discharge destination (home, nutritional rehabilitation unit)
- WAZ (≤ -3, > -3)
    - Target randomization is approximately 2/3 of participants with WAZ between -2 and -3 and approximately 1/3 with WAZ ≤ -3.

Blinding: This is a double-blind study. The placebo doses will be prepared to be as similar as possible in appearance and odor to Bi-26. The final packaging material and the final target fill weight for Bi-26 and placebo will be identical.

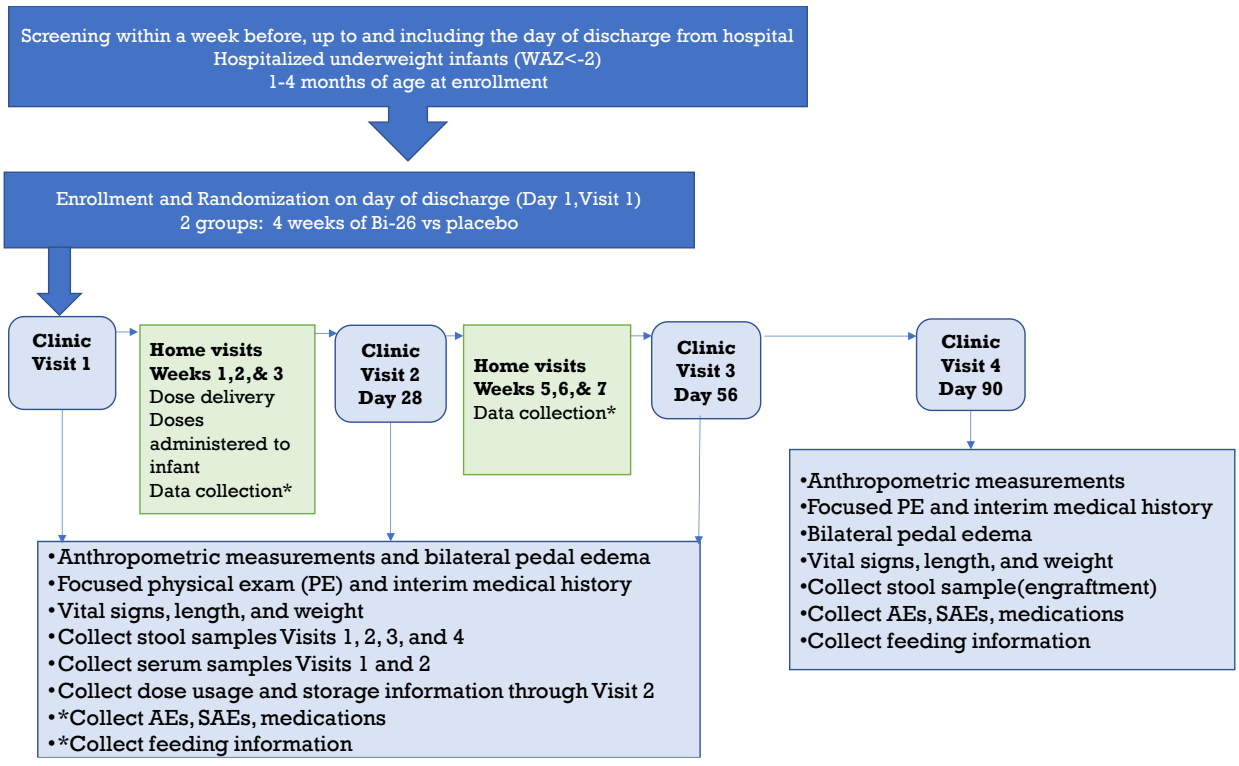
Total duration of study participation: The study duration for each participant is approximately 3 months.

Study sites: The study will be conducted at 4 or more sites in 2 or more low-income countries.

Infant Feeding: Breast-feeding counseling as recommended by the WHO will be conducted by study staff.

Independent Data Monitoring Committee: An IDMC will be used in this study for safety review. Methods for unblinding of IDMC members and support staff will be outlined in an IDMC charter.

Figure 1: Main Study Schema



Note that screening may occur on the same day as enrollment and randomization (Day 1)

An optional sub-study will be initiated at Day 35 to monitor refrigerator temperatures (summarized below).

Optional Sub-study

Approximately 20% of the study population enrolled at each site will also be enrolled into a prospective, observational sub-study to gather information on the reliability of refrigeration in the participants' homes over a 2-week period. The monitoring device will be placed in the refrigerator after the study intervention period has completed.

At the Day 35 home visit, a box with a temperature data logger will be placed in the refrigerator used by the sub-study participant's parent/legal guardian, and collected from the refrigerator at the Day 49 home visit.

Table 1: Objectives and Endpoints

Objectives	Endpoints
Primary	
To evaluate the change in weight (standardized for age) of infants receiving Bi-26	WAZ change from baseline (Day 1) to the Day 56 visit in Bi-26 group
Key Secondary	
To evaluate the change in weight of infants receiving Bi-26	Change in weight (in grams) from baseline (Day 1) to the Day 56 visit in the Bi-26 group
Other Secondary	
To estimate the treatment response over time associated with Bi-26	WAZ change from baseline (Day 1) over time through the Day 90 visit (longitudinal assessment) by duration of dosing
To assess the proportion of infants who achieve a specified change in WAZ from baseline to Day 56	Proportion of infants with a ≥ 0.3, ≥ 0.4, and ≥ 0.5 change in WAZ from baseline (Day 1) to Day 56 visit
To assess the proportion of infants who achieve a specified WAZ at Day 56	Proportion of infants who achieve a WAZ > -2 at Day 56 visit
To assess the re-hospitalization rate	Number of re-hospitalizations for acute non-surgical illness through the Day 56 visit
To assess the safety of Bi-26 supplementation through End of Study (EoS, Day 90 visit)	Number of adverse events (AEs) and serious adverse events (SAEs) through Day 90
To measure engraftment of *B. infantis* in participants	Presence of *B. infantis* in stool on Days 1, 28, 56, and 90

Objectives	Endpoints
Exploratory	
To describe stool biomarkers in response to Bi-26 supplementation in terms of stool composition (e.g., microbiome, pH)	Stool biomarkers on Days 1, 28, and 56
To describe the blood biomarkers (e.g., inflammation, metabolomics), in response to Bi-26 supplementation	Blood biomarkers on Days 1 and 28

Table 2: Schedule of Activities (SoA)

Activities	Screen	Clinic Visit 1	Home Visits			Clinic Visit 2	Home Visit	Home Visit	Home Visit	Clinic Visit 3	Clinic Visit 4	Discon Visit[b]
Visit window Days (D)	≤7 days before Day 1	**Day 1[a]**	**Wk 1 D 7**	**Wk 2 D 14**	**Wk 3 D 21**	**M 1 D 28**	**Wk 5 D 35**	**Wk 6 D 42**	**Wk 7 D 49**	**D 56 M 2**	**D 90 M 3**	
		±0	±1	±1	±1	±1	±2	±2	±2	±2	±2	
Informed consent (while in hospital) [c]	X											
Check inclusion and exclusion criteria /verify eligibility [d]	X	X										
Demographic characteristics	X											
Obtain estimation of socioeconomic status after enrollment		X										
Record medications for infant	X	X	X	X	X	X	X	X	X	X	X	X
Record antibiotics/probiotics taken by mother	X	X	X	X	X	X	X	X	X	X	X	X
Anthropometric measurements [e], bilateral pedal edema	X	X				X				X	X	X
Full medical history [f], full PE [f], VS, and L and W	X											
Interim history [g], focused PE [g] *as necessary*, VS, L and W		X				X				X	X	X
Randomization (at hospital discharge)		X										
Collect engraftment stool sample		X				X				X	X	
Collect exploratory stool samples [h]		X				X				X		
Collect serum samples [h]		X				X						
Provide a week's worth of doses for daily administration [i]		X	X	X	X							
Bi-26/placebo for daily administration [j]		X	X	X	X	X						
Record all AEs and SAEs	X	X	X	X	X	X	X	X	X	X	X	X
Provide feeding and dosing diary/review contents with parent(s)/legal guardian		X	X	X	X	X	X	X	X	X	X	
Record number of doses used from diary			X	X	X	X						X
Record feeding history (number of feedings that are from breastmilk, and from other source/s) from diary [k]			X	X	X	X	X	X	X	X	X	X
Record dose storage information			X	X	X	X						X

Activities	Screen	Clinic Visit 1	Home Visits			Clinic Visit 2	Home Visit	Home Visit	Home Visit	Clinic Visit 3	Clinic Visit 4	Discon Visit[b]
Visit window Days (D)	≤7 days before Day 1	**Day 1[a]**	**Wk 1 D 7**	**Wk 2 D 14**	**Wk 3 D 21**	**M 1 D 28**	**Wk 5 D 35**	**Wk 6 D 42**	**Wk 7 D 49**	**D 56 M 2**	**D 90 M 3**	
		±0	±1	±1	±1	±1	±2	±2	±2	±2	±2	
Sub-study activities [l]: • Place box with temperature data logger in the home refrigerator							X					
• Collect box with temperature data logger from the home refrigerator									X			

D= day; Wk= week, M=month; AE= adverse event; L= infant's length; PE = physical examination; VS= vital signs; W= infant's weight

[a] All study assessments related to determination of the participant's health (medical history, PE, VS. L and W, anthropometric measures, bilateral pedal edema) and collection of all study samples should occur prior to administration of the study intervention.

[b] Discon visit =A discontinuation visit will be scheduled, whenever possible, for participants who discontinue or withdraw from the study.

[c] Informed consent and the screening visit may occur the same day as enrollment and randomization (Day 1). Study assessments should not be duplicated for visits completed on the same day. Participants who are out of window due to a change in expected discharge date, may be rescreened, if applicable.

[d] Enrollment will occur on Day 1 after all eligibility criteria have been verified. Record whether discharged to home or nutritional rehabilitation unit at Visit 1.

[e] Anthropometric measurements: WAZ, weight-for-length z score (WLZ), mid-upper arm circumference (MUAC), head circumference, and skinfold thickness

[f] Refer to Section 8.3.1for full medical history and full PE

[g] Refer to Section 8.3.2 for interim medical history and focused PE

[h] Samples to measure exploratory biomarkers will be collected.

[i] Doses to be delivered within 2 days before planned use to ensure no break/no delay in dosing regimen.

[j] Monitor infants for 30 minutes after dosing, and a repeat dose will be administered if infant vomits within this observation period. If the daily dose is administered at the clinic during a visit, steps should be taken to ensure that blinding is maintained by not having any study personnel present during reconstitution and dose administration.

[k] The dosing information described in Section 6.4 (Feeding history) should be recorded in the diary from Day 1 through Day 28.

[l] Participants' parent/legal guardian will be asked if they agree to participate in this sub-study and consent obtained at the enrollment visit. Approximately 20% of the participants at each site will be enrolled into the sub-study. Boxes with a temperature data logger will be placed in the home refrigerators at the participants' homes and collected after 2 weeks. The study staff will return the box to the clinic so the information from the temperature data logger can be uploaded.

2. Introduction

Infant malnutrition, including undernutrition, can lead to impaired growth, immune and metabolic dysfunction, increased risk of infection, altered development of the central nervous system, and other abnormalities [Black 2008; Black 2013; Kerac 2015; Munirul Islam 2019]. Globally in 2020, 85 million children under 5 years of age were considered underweight (WAZ <-2 SD from median for WHO Child Growth Standards), the majority of whom lived in Asia and Africa [WHOa 2021]. As of 2020, the prevalence of underweight children under 5 years of age approached 40% in some low- and middle-income countries (LMICs) [WHOa 2021].

The burden of disease experienced by underweight children is significant, particularly in resource-poor countries. Undernutrition accounts for a global mortality of more than 3 million children every year [World Hunger Organization 2022] and around 45% of deaths among children under 5 years of age, most commonly in LMICs [WHOb 2021]. Underweight infants hospitalized with an acute illness are particularly at high risk of death. A meta-analysis from 23 studies of over 33,000 children under 5 years of age hospitalized with pneumonia found that moderately underweight and severely underweight children (defined in this study as WAZ between -2 and -3 and WAZ less than -3) were 2 and 4.6 times more likely to die, respectively, compared with children of normal weight [Kirolos 2021]. This analysis further estimated that over half of in-hospital child pneumonia deaths in LMICs were attributable to being underweight, with higher deaths in those severely underweight (median 40.9%, range 14.7 to 69.9) compared to those moderately underweight (median 18.3%, range 10.8 to 34.6).

Several studies have reported that in severely underweight children <5 years of age the inpatient and post-hospital discharge mortality rates are similarly high. A study in Bangladesh in children who also had pneumonia demonstrated an in-hospital mortality of 8.6% and mortality of 8.7% within 3 months of discharge [Chisti 2014]. Mortality in severely underweight children hospitalized in Zambia and Zimbabwe for treatment of complicated acute malnutrition was nearly 10% in the year following discharge [Bwakura-Dangarembizi 2021].

Mortality is also high in the subset of underweight children less than 6 months of age. In a study of underweight Kenyan infants less than 6 months of age, in line with what was observed in studies in older children, in-hospital and post-discharge mortality within 1 year were similar (4.9% and 5.3%, respectively) [Mwangome 2017], indicating that these infants remain vulnerable even after acute inpatient management has completed. In the Kenyan study, WAZ was identified as a better predictor of inpatient and post-discharge mortality than some other anthropometric indices traditionally used to assess nutritional status. These findings were reinforced by subsequent studies [Hoehn 2021;Chowdhury 2021] and support that WAZ should be used for early diagnosis of underweight infants and to inform patient management decisions.

Alterations in the gut microbiome have recently been strongly implicated in early childhood malnutrition and risk for intestinal inflammation [Kane 2015]. A healthy infant gut microbiome, dominated by *Lactobacillus* and *Bifidobacterium,* may contribute to metabolic functions that are essential to infant growth and development, such as development of the immune system, host metabolism and colonization resistance to enteric pathogens. Gut dysbiosis, an imbalance in microbial composition characterized by the overrepresentation of potentially pathogenic taxa, is implicated in mediating persistent pathophysiological and immune abnormalities [Casaburi

2021] and is thought to play a role in nutrient malabsorption leading to underweight infants and failure to thrive [Duar 2020; Casaburi 2021].

Existing therapies for infants that promote growth do so by delivering the requisite nutrients but do not address imbalances in gut bacteria that are critical for digesting and absorbing food and preventing pathogens from overgrowing and translocating. Children hospitalized for malnutrition are also most often treated with a regimen of antibiotics which can further disrupt the healthy microbes that protect from infection and establish a self-reinforcing cycle. With the growing recognition of the association between the gut microbiome and health, there has been an increasing focus on exploring products that are capable of improving health beyond providing basic nutrition and address the imbalance in the gut microbiome. Probiotics are defined as live microorganisms that, when administered in adequate amounts, confer a health benefit on the host [Hill 2014]. Bifidobacteria are common probiotic agents.

The presence and dominance of bifidobacteria in the microbiota of the infant gut, as well as the *Bifidobacterium* composition at a species level, changes over time with relative abundance decreasing throughout development due to weaning and /or introduction of solid foods [Arrieta 2014]. Breast-feeding supports growth of a healthy gut flora including *Bifidobacterium* strains that metabolize HMOs while supplemental feeding with formula is associated with less *Bifidobacterium* dominance [Arrieta 2014].

Bifidobacterium longum subspecies infantis (*B. infantis*) is a commensal bacterial strain common in the microbiome of breast-fed infants younger than 6 months of age in LMICs that has the unique ability to efficiently process HMOs. Although breast-feeding support is a key part of treatment of malnourished infants, this alone may be insufficient to support recovery. A decrease in abundance or absence of *B. infantis* could lead to inadequate HMO processing, elevating intestinal pH and increasing the risk of pathogen overgrowth. Intervention with a *B. infantis* probiotic strain such as Bi-26, in vulnerable malnourished infants could have a significant short-term impact on nutritional recovery and mortality, and long-term impact on health and development outcomes by potentially breaking the cycle of impaired immunity, recurrent infections, and worsening malnutrition.

2.1. Background and Study Rationale

Bifidobacteria are gram-positive, heterofermentative, anaerobic bacteria that colonize the newborn gut within the first days and weeks of life. They are often the most abundant bacterial genus in a breast-fed infant's intestinal flora. *B. infantis* metabolizes HMOs and produces short chain fatty acids (SCFA) that are thought to play an important role in nutrition and immune development, stimulating anti-inflammatory and inhibiting pro-inflammatory responses [Halloran 2019; Henrick 2021].

B. infantis Bi-26 strain is a nutritional probiotic intervention that will be evaluated in this trial for its ability to improve weight gain and health outcomes of underweight infants with WAZ <-2 who have been hospitalized with acute illness.

Clinical study data in the United States (US) have shown that *B. infantis* strain EVC001 colonizes the gut and is associated with significant changes to fecal microbiome composition in healthy breast-fed infants [Frese 2017; Henrick 2019]. Supplementation of *B. infantis* EVC001

has been associated with a reduction in pro-inflammatory cytokines [Henrick 2019]. Similar observations have been reported for preterm infants given *B. infantis* EVC001 [Nguyen 2021].

In a pilot trial evaluating *B. infantis* EVC001 supplementation in Bangladesh in hospitalized 2- to 6-month-old infants with SAM, i.e., weight-for-length z score [WLZ] <-3, researchers observed that daily supplementation with *B. infantis* for 4 weeks was associated with *B. infantis* colonization and an increase in WAZ [Barratt 2022].

A number of *B. infantis* strains, including Bi-26, have been designated as generally recognized as safe (GRAS) based on review by a panel of independent experts in the field. Furthermore, the US Food and Drug Administration (FDA) did not have questions about the GRAS determination of Bi-26 (GRN 985). In the US, the GRAS determination and the FDA no question letter allow the use of Bi-26 under its intended conditions of use (healthy term infants) in infant formula. The strain bearing the GRAS designation can also be used in dietary supplements, including those for infants.

In low-income countries, *B. infantis* strain Bi-26, evaluated in this study, could potentially be used as a dietary supplement in underweight infants if the safety and efficacy findings of the previous pilot study are confirmed.

2.2. Benefit/Risk Assessment

B. infantis supplements, including Bi-26 strain, that are designated as GRAS are considered safe for consumption in healthy term infants that consume breastmilk. Evidence supports that Bi-26 displays common probiotic characteristics, and the history of *B. infantis* used in food, as well as clinical trials at the species level, have been reviewed to support the safety assessment of the Bi-26 strain (International Flavors & Fragrances Inc. Bi-26 Investigator's Brochure).

Similar products to Bi-26 have been used in very vulnerable populations with increased intestinal permeability such as very low birth weight premature infants, with no safety signals detected [Garland 2011].

A Cochrane review of 24 trials of probiotic supplement administration for the prevention of necrotizing enterocolitis (NEC), late-onset sepsis and death in preterm infants noted that none of the studies reported systemic infection associated with probiotic use [AlFaleh 2014]. In this review, the majority of reported short-term adverse consequences associated with probiotic administration are limited to individual case reports of bacteremia a decrease in all-cause mortality, and no increased risk of culture-proven sepsis, and no sepsis due to probiotic species [Fleming 2019].

A large randomized controlled trial of a different supplement containing *B. infantis* in preterm infants under 1500g birth weight did not detect any safety signals and found reduced rates of NEC in the active treatment group [Jacobs 2013].

No safety concerns have been reported after *B. infantis* supplementation evaluated in infants and adults [Chichlowski 2020; Smilowitz 2017]. Stool *Bifidobacterium* spp. count was significantly higher and stool frequency significantly lower in infants receiving *B. infantis* and no differences in health and safety outcomes were detected between infants receiving *B. infantis* and lactation support, compared to those only receiving lactation support. One month after discontinuing feeding *B. infantis*, stool count of *B. infantis* persisted and was significantly higher compared to

the control group [Frese 2017]. The dominance of *B. infantis* influenced beta diversity (diversity between samples). However, there were no differences in terms of microbial species richness (alpha diversity) as the Shannon diversity index was similar between control and *B. infantis* groups. Lack of differences in alpha diversity between control and *B. infantis* is consistent with previous reports on breast-fed infants. Brink, et al, reported lower alpha diversity in infants receiving human milk compared to infant formula [Brink 2020].

Of note, the quality control of the study intervention will be important since contamination is a potential known risk for this type of product.

Bacterial transmigration and bacteremia have been described in case reports of *B. infantis* products but are considered coincidental, and not pathogenic. No bacterial transmigration or bacteremia cases have been reported with Bi-26 in ongoing infant clinical trials.

Potential benefits of supplementing infant feeding in low-income countries with *B. infantis* include a possible reduction in gut inflammation, and improved nutrient adsorption, potentially resulting in improved health outcomes. The findings from the SYNERGIE study with *B. infantis* strain EVC001 support the potential for positive clinical outcomes with *B. infantis* supplementation [Barratt 2022]. In the SYNERGIE study, following hospital discharge and acute phase management, infants were transferred to nutritional rehabilitation units and randomized to receive either 8 billion CFUs (target) of *B. infantis* EVC001 (n=20), 8 billion CFUs (target) or EVC001 plus probiotic LNnT (n=21), or lactose placebo (n=21). Infants received 4 weeks of supplementation and were followed for an additional 4 weeks for outcomes. Researchers observed that daily supplementation with *B. infantis* for 4 weeks was associated with *B. infantis* colonization and an increase in anthropometric measures, including WAZ [Barratt 2022]. This study provides support for a larger study assessing weight gain in underweight infants following supplementation with other *B. infantis* strains.

3. Objectives and Endpoints

See Table 1.

4. Study Design

4.1. Overall Design

Disclosure statement: This is a Phase 3, randomized, double-blind, placebo-controlled study to demonstrate increased weight gain with Bi-26 supplementation versus placebo in underweight infants (WAZ<-2) discharged from hospital following acute illness and to evaluate the safety of Bi-26 supplementation in this population.

Underweight infants (WAZ<-2), who are between 1 and 4 months of age at enrollment and are currently hospitalized for acute non-surgical illness, will be enrolled to receive daily supplementation of Bi-26 or placebo for 28 days with a total study duration of approximately 90 days.

Intervention: Refer to Section 6.

Study Population: Refer to Section 5.

Screening: Screening will take place during the infant's hospitalization, within a week before anticipated hospital discharge. A participant discharged to a nutrition rehabilitation unit would still be considered a hospital discharge.

Enrollment and Randomization: Enrollment and randomization will take place on the day of hospital discharge (Day 1) and after the infant participant has completed the acute stabilization phase of treatment, including fluid rehydration and antibiotic course as needed.

Note: Screening may be conducted on the same day as enrollment/randomization (Day 1). Study assessments should not be duplicated if screening and Day 1 occur on the same day.

Blinding: Refer to Section 6.2.

Total duration of study participation: The study duration for each participant is approximately 3 months.

Study sites: The study will be conducted at 4 or more sites in 2 or more low-income countries.

Infant Feeding: Breast-feeding counseling as recommended by the WHO will be conducted by study staff.

IDMC: Refer to Section 8.4.5.

Optional sub-study: After the 28-day intervention period has completed, participants may be enrolled into an optional, descriptive sub-study to gather information on the reliability of refrigeration in the participants' homes. Refer to Section 4.5.

4.2. Scientific Rationale for Study Design

Gut dysbiosis, or disrupted development of the gut microbiota, is hypothesized to be a contributing cause of infant malnutrition. Absolute *B. infantis* abundance is reported to be markedly lower in 3-to-24-month-old Bangladeshi infants and children under 24 months of age with SAM, compared to their healthy controls. The primary hypothesis to be tested in this study is Bi-26 supplementation for 28 days will lead to increased weight gain in underweight infants (WAZ <-2) who were hospitalized with acute non-surgical illness.

This study is designed as a double-blind, placebo-controlled study to enable equal attention and an unbiased evaluation of AEs and health outcomes.

WAZ was selected as the primary endpoint because it appears to be a better surrogate for mortality than WLZ [Mwangome 2019; Chowdhury 2021]. However, weight-for-age Z-scores require that the birth date of the infants enrolled is known and that weight is determined accurately.

We anticipate that an accurate gestational age at birth will often not be known for the infants enrolled. The study population will be heterogeneous and will include prematurely born infants and infants with normal weight-for-gestational age, infants with intrauterine growth retardation born small-for-gestational age, and infants with insufficient postnatal weight gain or weight loss due to illness and/or undernutrition.

4.3. Justification for Dose

The primary goal of this study is to confirm the findings of the initial study in Bangladesh (SYNERGIE study) in which 4 weeks of single daily feeding with *B. infantis* EVC001 resulted in significant weight gain relative to placebo, and in *B. infantis* colonization for at least 4 weeks post end of treatment. The target dose listed in the SYNERGIE study was approximately 8 billion CFUs with a 28-day duration of treatment; however, the amount of product initially packaged, and the potential range of doses administered over the course of the study was not provided [Barratt 2022]).

[redacted]. Thus, the anticipated range of CFU per dose included in this study is expected be generally comparable to that in the SYNERGIE study. Refer to the Gates MRI IB addendum for additional information.

4.4. End of Study Definition

A participant is considered to have completed the study if he/she completes the last study visit at Day 90.

The EoS is defined as the date of the last visit of the last participant in the study.

4.5. Optional Sub-study

The aim of this optional, prospective, observational sub-study is to collect information on reliability of refrigeration in the geographic areas where the study is being conducted and where *B. infantis* supplementation may be recommended by WHO if supported by clinical data.

Ensuring universal access to affordable, reliable, sustainable, and modern energy by 2030 is a sustainable development goal. Access to sufficient electricity is essential to reduce poverty as well as improve public services, such as healthcare. While strides have been taken to decrease the number underserved, as of 2020, 733 million people did not have access to electricity in 2020, over 75% (568 million people) of whom lived in sub-Saharan Africa [World Bank 2022]. Per estimates from a year prior, another 1 billion people live with unreliable or insufficient electricity [Sanni 2019].

Electricity deficits are greater in rural areas compared to urban areas [World Bank 2022]. In order to get a geographical cross-representation in this study, each clinical site will be asked to enroll up to 20% of their overall study population into the sub-study.

The selected participants will receive a box containing a temperature data logger to store in the home refrigerator. Participants may refuse enrollment into the sub-study without effecting their participation in the main study.

The box with the temperature data logger will be placed in the refrigerator used by the participant's parent(s)/legal guardian after the participant has completed the study intervention period and during the home visit on Day 35. The box will be collected from the refrigerator at the home visit on Day 49. Participants in the sub-study should keep the box unopened in the refrigerator until the box is collected by the study staff. The study staff will return the box to the clinic so the information from the temperature data logger can be uploaded.

No formal statistical analysis is planned; data will be presented descriptively.

5. Study Population and Eligibility Criteria

A sufficient number of participants will be screened to randomize approximately 396 study participants. Screening will take place while the infant is hospitalized.

Randomization will take place on the day of hospital discharge. Prospective approval of protocol deviations to recruitment and enrollment criteria, also known as protocol waivers or exemptions, is not permitted.

The preferred method of reconstitution of the study intervention is in breastmilk and therefore study activities are assigned to the mother of the infant participant. However, other caretakers may perform certain study activities (e.g., picking up the study doses, assisting in completion of the feeding diary, etc.).

5.1. Inclusion Criteria

Participants are eligible to be included in the study only if all of the following criteria apply:

Age

1. Participant must be between 30 days and 120 days of age (inclusive), at the time of enrollment (study Day 1)

Type of Participant and Disease Characteristics

2. Hospitalized for acute non-surgical illness
3. Completed acute stabilization phase of treatment, including fluid rehydration and antibiotic course, prior to enrollment (study Day 1)

Weight

4. WAZ at enrollment (study Day 1) is less than negative 2 (<-2)

Sex

5. Any

Informed consent

6. Participant's parent(s)/legal guardian is capable of giving informed consent which includes agreement to comply with the requirements and restrictions listed in the informed consent form (ICF) and in this protocol

Additional requirements

7. Participant's parent(s)/legal guardian agrees to stay in contact with the study site for the duration of the study, provide updated contact information as necessary, and have no current plans to relocate from the study area for the duration of the study
8. Participant's parent(s)/legal guardian has easy access to reliable refrigeration (for storage of investigational product)
9. Participant receives some feedings from breastmilk and mother intends to continue breast-feeding.

5.2. Exclusion Criteria

Participants are excluded from the study if any of the following criteria apply:

1. Congenital condition (suspected or confirmed) that the investigator considers likely to interfere with feeding or with normal growth and development
2. Infant has not been discharged from hospital since birth or has not been at home for at least one week since birth
3. Infant hospitalized with septic shock during current hospitalization
4. Infant required mechanical ventilation during current hospitalization
5. Infant with acute kidney injury on hospital admission
6. Infant with severe jaundice and suspected kernicterus
7. Infant receiving treatment for suspected or confirmed tuberculosis, or suspected or confirmed HIV infection
8. Ongoing infant antibiotic (e.g. as prophylaxis in sickle cell disease) and/or probiotic usage
9. Ongoing maternal antibiotic and/or probiotic usage for breast-feeding infants
10. Inability of participant's parent(s)/legal guardian to comply with protocol requirements, as per investigator assessment.

5.3. Screen Failures

A screen failure participant is defined as a participant whose parent(s)/legal guardian consented to participate in the clinical study, but the infant is not subsequently randomly assigned to group and study intervention on the Day 1 visit.

A minimal set of screen failure information is required to ensure transparent reporting of screen failure participants to meet the Consolidated Standards of Reporting Trials (CONSORT) publishing requirements and to respond to queries from regulatory authorities. Minimal information includes demography, screen failure details, eligibility criteria, and any SAE.

Infants who do not meet the eligibility criteria for participation in this study (screen failures) will not be rescreened. Infants whose screening visit is outside of the 7-day planned window, due to a delay in hospital discharge, may be rescreened and enrolled if all the eligibility criteria are met.

6. Study Intervention

Study intervention is defined as any investigational intervention, marketed product or placebo, intended to be administered to a study participant according to the study protocol. The study intervention includes Bi-26 and placebo, given to approximately 198 participants, per group.

All participants will be given either Bi-26 supplementation, or product matched placebo (lacking the active ingredient), based on randomization (refer to Section 6.2). A single dose (Bi-26 or placebo control) will be resuspended, and administered to each participant, daily, for 28 days. Participants will be monitored for 30 minutes, and a repeat dose will be administered if a participant vomits within this observation period.

While a total of 7 doses are to be administered to the participant per week for a total of 4 weeks, 2 additional doses will be supplied each week to account for repeat dose administration in the event of vomiting, or unexpected events which may render a dose unusable (e.g., spillage or otherwise compromised). Therefore, mothers will be dispensed 9 doses containing Bi-26 or placebo once a week, within 2 days before the doses are needed to ensure that doses are available when needed. Doses will either *1)* be delivered to the mother by study staff, or *2)* stored by staff, at the local health center to be picked up from the health center.

The product is presented as a lyophilized powder that the mother mixes with approximately 3 mL to 5 mL of breastmilk once daily and administers to the infant orally using a feeding syringe. If the mother is unable to express breastmilk, the powder may be mixed in approximately 3 mL to 5 mL of water. The participant's mother should provide the dose with a feeding at approximately the same time each day.

The study staff will counsel the mother on recommended breast-feeding practices as per WHO guidance and encourage the participant's mother to continue breast-feeding during the study.

The participant's parent(s)/legal guardian will also be counseled on the importance of having access to reliable refrigeration and to contact the study staff if there is ever a problem with product storage.

Further information on the study intervention dose and handling can be found in the Study Manual and Investigator's Brochure.

As noted in Section 5, because the preferred method of reconstitution of the study intervention is in breastmilk, study activities are assigned to the mother of the infant participant. However, other caretakers may perform certain study activities (e.g., picking up the study doses, assisting in completion of the feeding diary, etc.).

Table 3: Study Intervention

Group Name	Bi-26	Control
Intervention Name	*Bifidobacterium longum* ssp. *infantis** Bi-26™ strain Product code MNK01	Placebo
Type	Dietary supplement, presented as lyophilized powder	Product matched lyophilized powder lacking *B. infantis*
Dose Formulation	Doses consist of a powder that the mother mixes with approximately 3 mL to 5 mL of breastmilk or water	Powder that the mother mixes with approximately 3 mL to 5 mL of breastmilk or water
Unit Dose Strength	Contains approximately 5 billion CFU of *B. infantis* at end of shelf-life, with potato maltodextrin as the excipient, [redacted]	Contains potato maltodextrin as the excipient
Dosage	A single dose per day	A single dose per day
Route of Administration	Oral, using a feeding syringe, given directly into the infant's mouth	Oral, using a feeding syringe, given directly into the infant's mouth
Packaging and Labeling	Provided as single doses, labeled per country requirement.	Provided single doses, labeled per country requirement.
Current/Former Name or Alias	Not applicable	Not applicable

*Family: *Bifidobacteriaceae*, Genus: *Bifidobacterium*, Species*: longum*, Subspecies: *infantis (B. infantis),* Strain Bi-26

6.1. Handling, Storage, and Accountability

Each participant's mother will be dispensed 9 single doses of the study intervention (either Bi-26 or placebo) once a week for a total duration of 4 weeks.

Only authorized site staff may supply study intervention. Doses will either be delivered to the participant's mother by study staff, or be stored by study staff at the local health center, to be picked up from the health center.

The preparation of study interventions will be demonstrated to the participant's mother at the first study visit and written instructions with images will be provided to each participant's mother. These instructions will be provided to the sites in the Study Manual.

Doses must be shipped and stored at 2°C to 8°C after delivery to the local health center or home. The investigator or designee must confirm appropriate temperature conditions have been

maintained during transit for all study intervention received and any discrepancies are to be reported and resolved before use of the study intervention.

All study intervention taken to the local health center must be stored in a secure, controlled environment, and should be monitored (manual or automated) in accordance with the labeled storage conditions, with access limited to the authorized site staff. Monitoring will be addressed in the Study Manual.

All study intervention delivered to the participant's home, must be stored in a refrigerator at the participant's or neighbor's home with confirmation from the parent(s)/legal guardian that the electricity in the home is reliable for the duration of the study.

The participants' parent(s)/legal guardian will be asked to inform the site if refrigeration was off or unreliable at any time during dose storage, and for how long, and for how many of the doses.

The investigator or designee is responsible for study intervention accountability, reconciliation, and record maintenance (i.e., receipt, reconciliation, and final disposition records).

Further guidance and information for the final disposition of unused study interventions are provided in the Study Manual.

6.2. Measures to Minimize Bias: Randomization and Blinding

Randomization: On the day of hospital discharge (Visit 1, Day 1) participants will be randomized 1:1 to receive either Bi-26 or placebo. To minimize differences between Bi-26 and placebo groups within a given treatment arm, randomization will be stratified by:

1) country
2) age at enrollment (Study Day 1):30-59 days of age, 60-120 days of age
3) discharge destination (home, nutritional rehabilitation unit)
4) WAZ ($\leq$ -3 versus > -3)

Target randomization is approximately 2/3 of participants with WAZ between -2 and -3 and approximately 1/3 with WAZ $\leq$ -3. As such, a stratification blocking factor will be used in the ratio of 1:2 for (WAZ$\leq$-3):(-3< WAZ< -2) within each treatment arm. Dynamic randomization will be utilized to balance the treatment groups for the other stratification factors.

Blinding: The study is double-blind. The placebo doses will be prepared to be as similarly as possible in appearance and odor to Bi-26. The final packaging material and the final target fill weight for Bi-26 and placebo will be identical. Study participants, principal investigators and study site personnel will remain blinded to treatment assignments throughout the study. The Gates MRI Medical Monitor, study monitors, and any other Gates MRI and contract research organization (CRO) personnel who are regularly in contact with the study site will remain blinded. Selected individuals listed below will have access to treatment allocations while the study is blinded:

- Biostatistician preparing the randomization list
- Biostatistician preparing the IDMC data
- IDMC members.

Additionally, in the event of a suspected unexpected serious adverse reaction (SUSAR), designated members of the sponsor or the sponsor representative's pharmacovigilance department may be required to unblind a study participant to meet country-specific reporting requirements.

Emergency unblinding: All participants will be centrally assigned to randomized study intervention using an Interactive Web Response System (IWRS). Before the study is initiated, the login information and directions for the IWRS will be provided to each site.

The IWRS will be programmed with blind-breaking instructions. In case of an emergency, the investigator has the sole responsibility for determining if unblinding of a participants' treatment assignment is warranted. Participant safety must always be the first consideration in making such a determination. If the investigator decides that unblinding is warranted, the investigator should make every effort to contact the sponsor prior to unblinding a participant's treatment assignment unless this could delay emergency treatment of the participant. If a participant's treatment assignment is unblinded, the sponsor must be notified within 24 hours after breaking the blind. The date and reason that the blind was broken must be recorded in the source documentation and the electronic case report form (eCRF), as applicable.

6.3. Study Intervention Compliance

Participant compliance with study intervention will be assessed at each visit. Compliance will be assessed by counting returned doses, and by asking the mother if her infant received a dose each day, and if any of the additional doses were used and why. Deviations from the planned dosing regimen will be recorded in the eCRF.

The following data will also be collected at each visit specified in the SoA for study intervention storage information:

- where the study intervention is being stored (at the local health center or at the home, or a neighbor's home)
- any issues with study intervention storage (and how many doses were compromised).

6.4. Feeding History

The mother of the participating infant will be asked to complete a diary to record the number and types of feedings (e.g., breast-feeding, formula feeding, other) daily through Day 90.

The type of liquid (breastmilk or water) used to mix with the Bi-26 or placebo, whether the full dose was taken, and whether a repeat dose was administered will also be recorded in the diary for Days 1 through 28.

The completed diary should be collected by the site staff.

6.5. Concomitant Therapy

Any medication or vaccine (including over-the-counter or prescription medicines, vitamins, and/or herbal supplements) that the participant is receiving at the time of screening or receives during the study must be recorded at the scheduled timepoints along with:

- Reason for use
- Dates of administration including start and end dates
- Dosage information including dose and frequency.

Any maternal use of antibiotics or probiotics should be captured through Day 90.

The Medical Monitor should be contacted if there are any questions regarding concomitant or prior therapy.

6.6. Dose Modification

Dose modification is not planned.

7. Discontinuation of Study Intervention and Participant Discontinuation/Withdrawal

7.1. Discontinuation of Study Intervention

In rare instances, it may be necessary for a participant to permanently discontinue study intervention earlier than the protocol planned duration. Stopping the study intervention may be initiated by either the participant's parent(s)/legal guardian or the Investigator. The Investigator should discontinue the study intervention if the participant experiences intolerable AEs, or if the Investigator believes that continuing dosing would result in an unfavorable risk/benefit for the trial participant. If study intervention is permanently discontinued, the participant will remain in the study to be evaluated for safety and weight change.

Refer to the SoA (Table 2) for data to be collected at the time of discontinuation of study intervention for any reason.

7.2. Participant Discontinuation/Withdrawal from the Study

A participant may withdraw from the study at any time at the request of the participant's consenting parent/legal guardian, or may be withdrawn at any time at the discretion of the investigator for safety, or other reasons. This is expected to be uncommon.

At the time of discontinuing from the study, if possible, an early discontinuation visit should be conducted, and data should be collected, if possible, as shown in the SoA (Table 2). The participant will be permanently discontinued both from the study intervention and from the study at that time.

If the participant's consenting parent/legal guardian withdraws consent for disclosure of future information, the sponsor may retain and continue to use any data collected before such a withdrawal of consent.

If a participant's parent/legal guardian withdraws participant from the study, they may request destruction of any samples taken and not tested, and the investigator must document this in the site study records.

7.3. Replacement of Participants

Participants prematurely discontinued from the study will not be replaced.

7.4. Lost to Follow-up

A participant will be considered lost to follow-up if the participant's parent(s)/legal guardian repeatedly fails to return for scheduled visits, whether at the clinic or at home, and is unable to be contacted by the study site.

The following actions must be taken if a participant's parent(s)/legal guardian fails to return to the clinic for a required study visit or is not at home when a home visit is scheduled, and the parent(s)/legal guardian cannot be found for the home visit:

The site must attempt to contact the participant's parent(s)/legal guardian and reschedule the missed visit as soon as possible and counsel the participant's mother on the importance of maintaining the assigned visit schedule and ascertain whether the participant's mother wishes to and/or should continue in the study.

Before a participant is deemed lost to follow-up, the investigator or designee must make every effort to regain contact with the participant's parent(s)/legal guardian (where possible, 3 telephone calls and, if necessary, a certified letter to the participant's parent(s)/legal guardian last known mailing address or local equivalent methods). These contact attempts should be documented in the participant's medical record.

Should the participant's parent(s)/legal guardian continue to be unreachable, the participant will be considered to have withdrawn from the study.

If the participant's parent(s)/legal guardian is unreachable when the weekly doses are being delivered at the home visit, and the doses are not received, the investigator or designee must make every effort to regain contact with the participant's parent(s)/legal guardian (as mentioned above) to deliver the doses.

8. Study Assessments and Procedures

In this study, visits will be comprised of both clinic visits and home visits, as shown in the SoA (Table 2). Home visits and all assessments at these visits will be performed by trained study team staff.

Screening procedures will be conducted after informed consent is obtained (refer to Section 10.1.3) and within 7 days before anticipated hospital discharge and enrollment/randomization (Day 1). If informed consent and screening activities are conducted on the same day as enrollment/randomization (Day 1), assessments should not be duplicated. All screening evaluations must be completed and reviewed to confirm that potential participants meet all eligibility criteria. The investigator will maintain a screening log to record details of all participants screened and to confirm eligibility or record reasons for screening failure, as applicable.

On Day 1, after all eligibility criteria are verified, eligible participants will be enrolled and randomized. Randomization should take place at the time of hospital discharge. Refer to Section 6.2 for randomization.

Procedures conducted as part of the participant's routine clinical management (e.g., procedures while hospitalized) and obtained before the ICF is signed, may be utilized for screening or baseline purposes provided the procedures met the protocol-specified criteria and were performed within the time frame defined in the SoA (Table 2).

Adherence to the study design requirements, including those specified in the SoA is essential and required for study conduct.

Immediate safety concerns should be discussed with the sponsor immediately upon occurrence or awareness to determine if the participant should continue or discontinue study intervention.

Study procedures and their timing are summarized in the SoA (Table 2). Protocol waivers or exemptions are not allowed.

8.1. Demographic and Baseline Characteristics

The following information pertaining to the participant will be obtained from the participant's mother at screening:

- Participant's sex, race, ethnicity, date of birth (including day, month, and year), and whether the delivery was a C-section.
- Participant's gestational age in weeks, if known, and the participant's birth weight (in kilograms). This information may be obtained from the hospital medical record if necessary.
- The mother's age will be obtained whenever possible.
- The participant's mother will be asked about breast-feeding practices from the time of the participant's birth, and planned feeding practices during the study (and this information will be captured in the case report form).

A full medical history and full physical examination will be performed at the screening visit (refer to Section 8.3.1). The participant's anthropometric measurements will be taken at the screening visit and at other specified timepoints in the SoA (refer to Table 2 and Section 8.3.1for more details).

If the eligibility criteria are met, the participant will be enrolled into the study on Day 1. After enrollment, an estimation of socioeconomic status will be obtained if possible.

Hospital discharge to home, or to nutritional rehabilitation unit, will be collected on Day 1 and obtained from either the participant's parent(s)/legal guardian, or the hospital medical record. The anthropometric measurements obtained at Day 1 will be considered baseline for the purposes of statistical analyses (refer to Section 9.2).

8.2. Efficacy Assessments

Efficacy assessments include calculation of the WAZ and assessments for re-hospitalizations for acute non-surgical illness at various time points per SoA (Table 2).

Refer to Section 9.7 for analyses.

8.3. Safety Assessments

Safety assessments include SAEs and AEs from the time the ICF is signed through Day 90 (or at the last visit [Discontinuation Visit]), if possible, if participant is discontinued/withdrawn early). Planned time points are provided in the SoA (Table 2).

Any AEs and any SAEs that occur from the time of screening until Visit 1 may be obtained from the participant's hospital records.

Refer to Section 8.4 for details regarding AEs and SAEs.

Refer to Section 9.7.1for analyses.

8.3.1. Medical History and Full Physical Examination

A full medical history, including medications and vaccinations received, will be recorded at screening to assess enrollment eligibility and to provide a baseline.

All conditions that exist before and during screening will be recorded in the medical history. The infant's HIV status, including history of HIV exposure, will also be recorded. Physical examination at screening will include, at a minimum, assessment of all planned anthropometric measurements, including weight to assess WAZ, length to measure WLZ score, MUAC, head circumference, and skinfold thickness. Details regarding standardized anthropometric measurements will be provided in the Study Manual. Presence of bilateral pedal edema will also be assessed.

In addition, resting vital signs, including body temperature (refer to Section 8.3.3) and other assessments of eligibility criteria requiring a physical examination will be performed.

A complete physical examination will include, at a minimum, assessments of the head, eyes, ears, nose and throat, cardiovascular, respiratory, gastrointestinal, musculoskeletal, and neurological systems.

8.3.2. Interim History and Focused Physical Examination

The interim medical history covers the period since the previous clinic visit. If the interim history raises any concerns, a focused physical examination will be conducted as appropriate if indicated by participant's medical issues and will include assessments of body systems involved. AEs identified as a result of the review of the interim medical history should be recorded as outlined in the SoA (Table 2).

8.3.3. Vital Signs

Vital signs, length and weight will be assessed and recorded at all clinic visits.

Vital sign measurements will include pulse rate, pulse oximetry, and body temperature.

Measurements are to be repeated if clinically significant changes are observed or a machine error occurs.

Body temperature should be taken per standard of care at the site. If the participant passes a bowel movement during temperature collection at a clinic visit only, this stool sample should be collected for biomarkers (see Section 8.6).

8.4. Adverse Events, Serious Adverse Events

The definitions of an AE or SAE can be found in Section 10.2.

AEs and SAEs will be assessed as further described in this section and recorded at timepoints indicated in Table 2. The infant's parent(s)/legal guardian will be asked about any event that occurred since the previous clinic or home visit.

The investigator and any qualified designees are responsible for detecting, documenting, and recording events that meet the definition of an AE or SAE and remain responsible for following up AEs that are serious, considered related to the study intervention or study procedures, or that caused the participant to discontinue the study intervention or the study (see Section 7).

The investigator is solely responsible for assessment, including assignment of causality and intensity, reporting and management.

8.4.1. Time Period and Frequency for Collecting AE and SAE Information

All AEs and SAEs will be collected from the time the informed consent is signed through the EoS (Day 90 visit or at the Discontinuation Visit, as applicable). All AEs and SAEs will be classified according to the *Medical Dictionary for Regulatory Activities* (MedDRA).

All SAEs will be recorded and reported to the sponsor or designee immediately within 24 hours, as indicated in Section 10.2.4. The investigator will submit any updated SAE data to the sponsor within 24 hours of it being available.

Investigators are not obligated to actively seek AE or SAE information after conclusion of the study participation. However, if the investigator learns of any SAE, including a death, at any time after a participant has been discharged from the study, and if the investigator considers the event to be reasonably related to the study intervention or study participation, the investigator must promptly notify the sponsor.

8.4.2. Method of Detecting AEs and SAEs

The method of recording, evaluating, and assessing causality of AEs and SAEs and the procedures for completing and transmitting SAE reports are provided in Section 10.2.

Care will be taken not to introduce bias when detecting AEs and/or SAEs. Open-ended and nonleading verbal questioning of the participant is the preferred method to inquire about a SAE occurrence.

Day-to-day fluctuations in chronic conditions that were present at baseline, and do not represent a clinically significant change in the participant's health status will not necessarily be reported as AEs. If the investigator determines that this change is related to the study intervention it will be reported as an AE.

8.4.3. Follow-up of AEs and SAEs

After the initial AE/SAE report, the investigator is required to proactively follow each participant at subsequent visits. All SAEs will be followed until resolution, stabilization, the event is otherwise explained, or the participant is lost to follow-up (as defined in Section 7.4). Further information on follow-up procedures is provided in Section 10.2.3.

8.4.4. Regulatory Reporting Requirements for SAEs

Prompt notification by the investigator to the sponsor of a SAE is essential so that legal obligations and ethical responsibilities toward the safety of participants and the safety of a study intervention under clinical investigation are met. Refer to Section 10.2.4 regarding reporting timelines.

The sponsor has a legal responsibility to notify both the local regulatory authority and other regulatory agencies about the safety of a study intervention under clinical investigation. The sponsor will comply with country-specific regulatory requirements relating to safety reporting to the regulatory authority, Ethics Committees (ECs)/Institutional Review Boards (IRB), and investigators.

Investigator safety reports must be prepared for any SUSARs according to local regulatory requirements and sponsor policy and forwarded to investigators as necessary.

An investigator who receives an investigator safety report describing a SAE or other specific safety information (e.g., summary or listing of SAEs) from the sponsor will review it and will notify the EC/IRB, if appropriate according to local requirements.

8.4.5. Independent Data Monitoring Committee

The IDMC will operate according to a charter. The IDMC structure, participants and other details will be provided in the charter. The charter will be available prior to study start.

The role of the IDMC will be to review unblinded safety data and make recommendations to the sponsor. These recommendations may include continuing the study without modification, continuing the study with modifications, or terminating the study. Additionally, the sponsor will review data on an ongoing basis and may, on discussion with the IDMC, terminate the study if any clinically significant safety signal related to the study intervention is identified. The recommendations of the IDMC, along with the sponsor's decision, will be communicated to the investigators, the ECs/IRBs and the national regulatory authorities as required. The sponsor or its designee agrees to abide by any directives issued by the national regulatory authority or the EC/IRB.

8.5. Genetics

Genetics are not evaluated in this study.

8.6. Biomarkers

To assess the biomarker study endpoints, serum and stool samples will be collected from all infants for biomarker research. All of these samples, or a subset of these samples from a representative cohort of all participants, may be tested to identify analytes of interest.

At Visit 1 Day 1 and Visit 2 Day 28, as indicated in the SoA (Table 2), a blood sample will be collected and processed to serum from all participants. Restricted blood volumes may be dependent on the physician discretion and/or the participant's health/condition. Testing will be determined by the amount of available blood collected.

These serum samples will be tested for biomarkers including, but not limited to, host inflammatory biomarkers and metabolic analysis. No host genetic analyses will be performed.

The maximum amount of blood collected from each participant over the duration of the study will not exceed approximately 4 mL.

Repeat or unscheduled samples may be taken for safety reasons or for technical issues with the samples.

Stool samples for measurement of exploratory biomarkers will also be collected at clinic visits as specified in the SoA (Table 2), in addition to stool samples collected for the secondary endpoint, (refer to Section 9.7.1). Collection will be performed by trained study team staff at specified clinical visits. Either taking temperature or a rectal swab will be performed to stimulate defecation. Defecation at any time during the clinical visit prior to stool collection can substitute for this rectal swab.

Fecal pH and Bristol or similar stool scale will be measured and recorded by the trained study team staff, at the time of stool collection. Samples will be tested for microbiome composition including levels of total Bi-26 measured by nucleotide sequencing and/or polymerase chain reaction. Stool samples may be tested for biomarkers including, but not limited to, inflammatory biomarkers and host and/or bacterial metabolic analysis.

CCI

[redacted]

9. Statistical Considerations

9.1. General

All statistical analyses will be performed using SAS® (Version 9.3 or higher).

Analyses of continuous data will be summarized using descriptive statistics where the following parameters will be reported:

- number of observations
- number of missing observations
- mean
- median
- standard deviation (SD)
- minimum (min)
- maximum (max).

Categorical data will be presented with absolute and relative frequency (n and %).

For the summary statistics, the number of decimal places will be the same as for the source data for extreme values (min, max) and will exceed that of the source data by 1 for other parameters. Percentages will be presented with one decimal place and a percent sign (%).

All tests will be two-sided and performed at the two-sided 5% significance level if not otherwise specified. When reporting the results of significance tests p-values will be reported. All confidence intervals will be two-sided at the 95% level, unless otherwise stated.

All data will be presented in subject data listings.

Summary statistics will be presented separately for the treatment and follow-up periods.

9.2. Subject Disposition, Demographics and Baseline Data, Concomitant Medication, and Compliance

9.2.1. Subject Disposition

Number of subjects who were enrolled and randomized, took at least one dose of study supplement, completed the treatment phase, completed the follow-up phase, withdrew including reasons for withdrawal and the number of participants in each analysis set will be summarized. Protocol deviations will be listed.

9.2.2. Demographic and Baseline Data

The demographic, background, characteristics, and baseline data will be presented descriptively. Corresponding summaries will be presented for all participants enrolled.

9.2.3. Concomitant Medication

Medications will be coded by site of action and therapeutic and clinical characteristics using WHO's Anatomical Therapeutic Chemical (ATC) Classification, and will be presented descriptively

9.2.4. Compliance

Compliance will be presented descriptively. Compliance will be used in defining the per-protocol population.

9.3. Randomization

Participants will be randomized in a ratio of 1:1 to each of the 2 treatment arms, Bi-26 or placebo.

A stratification blocking factor will be used in the ratio of 1:2 for (WAZ $\leq$-3):(-3<WAZ<-2) within each treatment arm. Dynamic randomization algorithms will be used to balance treatment arms with respect to the other stratification factor levels (country, age at enrollment (Day 1), discharge destination) and ensure an overall treatment balance.

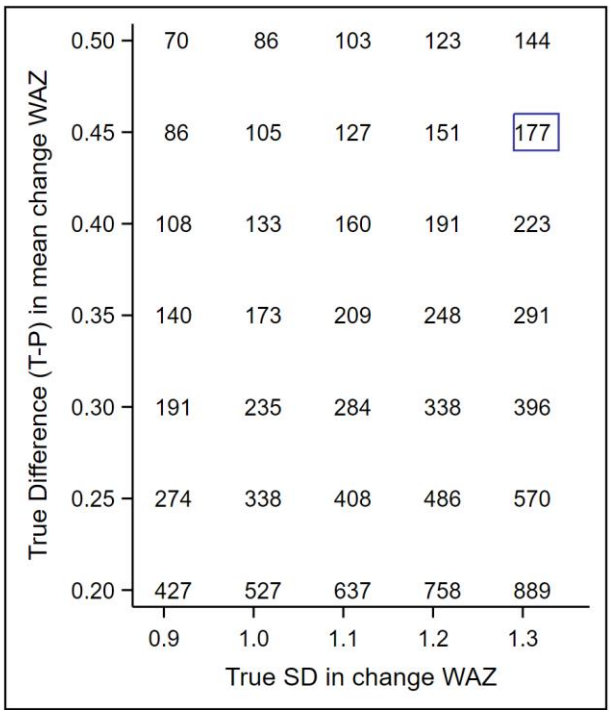
9.4. Primary Statistical Hypotheses and Sample Size Determination

The primary study hypothesis is that the difference in the mean change from baseline in WAZ is larger for participants randomized to at least 28 days of Bi-26 relative to placebo.

For the sample size calculation for the primary hypothesis, it is assumed that the between group difference in the WAZ change from baseline is 0.45 with a standard deviation of 1.3. Based on the above assumption(s) using a 2-sided alpha of 5% and a power of at least 90%, a total of 354 participants, 177 in each group, is required.

Assuming a 10% loss to follow-up and a 3% mortality rate, a sufficient number of participants will be screened to ensure approximately 396 randomly assigned participants are enrolled, 198 in each treatment group.

Figure 2: Sample size sensitivity



N/group, 1-sided α=2.5%, Power=90%

9.5. Populations for Analyses

For purposes of analysis, the following populations are defined:

- **Intention-to-treat (ITT) population**: All participants randomly assigned to study intervention.

- **Modified intention-to-treat (mITT) population**: All participants randomly assigned to study intervention, who received at least one dose (including a partial dose) of the study intervention. Participants will be analyzed according to the intervention they were randomized.

- **Per-Protocol (PP) population**: All participants randomly assigned to study intervention, who received the study intervention, have a Day 90 visit, and did not substantially deviate from the protocol procedures. Participants will be analyzed according to the intervention they actually received. Details of criteria for defining the PP population will be specified in the Statistical Analysis Plan (SAP). The inclusion of participants in the PP population will be determined prior to unblinding the study. A participant data listing and a table with protocol deviations will be presented.

- **Safety population**: All participants randomly assigned to study intervention, who received any dose, including a partial dose, of the study intervention. Participants will be analyzed according to the intervention they actually received.

9.6. Statistical Analyses

The principal features of the statistical analysis are described in this section. A more technical and detailed document of the features will be found in a separate SAP, which is to be finalized before unblinding of the study. When the study is completed and the data are collected, a blinded review of the planned analysis will be carried out. The review will look at the definition of outliers, violators, and exclusion of participants or data from the analysis sets. Decisions made based on the review will be documented.

9.7. Primary and Secondary Estimands

Participants randomized to the group receiving 28 days of Bi-26 will be compared to participants randomized to 28 days of placebo for the primary analysis.

Following International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH) E9 (R1) addendum, the estimands relating to the study objectives are constructed by using the 5 following attributes:

(a) treatment

(b) population

(c) variable/endpoint

(d) population level summary

(e) intercurrent events.

These attributes were specified to translate the study objective(s) into a treatment effect that is to be estimated (estimand). Refer to Table 4 and Table 5 for a summary of the primary and secondary estimands, respectively.

Table 4: Primary Estimands

Objective	Primary Estimand
To evaluate the change in weight (standardized for age) of infants receiving Bi-26	a. **Population**: The mITT population will be used for the analysis. b. **Endpoint**: WAZ change from baseline (Day 1) to the Day 56 visit in Bi-26 group. c. **Population level summary**: Mean change from baseline in WAZ in the Bi-26 group compared to placebo with stratification blocking factors and feeding regimen covariate(s). d. **Intercurrent event(s)**: Including but not limited to treatment discontinuation. *Further details will be specified in the SAP.*

Table 5: Secondary Estimands

Objective	Secondary Estimand
To evaluate the change in weight of infants receiving Bi-26	a. **Population**: The mITT population will be used for this analysis. b. **Endpoint**: weight change (in grams) from baseline (Day 1) to the Day 56 visit in Bi-26 group. c. **Population level summary**: Mean change from baseline in weight in the Bi-26 group compared to placebo with stratification blocking factors and feeding regimen covariate(s). d. **Intercurrent event(s):** Including but not limited to treatment discontinuation. Details will be provided in the SAP.
To estimate the treatment response over time associated with Bi-26	e. **Population**: The PP population will be used for the analysis. f. **Endpoint**: WAZ change from baseline (Day 1) over time through the Day 90 visit (longitudinal assessment) by duration of dosing. g. **Population level summary**: Mean change from baseline in WAZ over time in the Bi-26 group compared to placebo with stratification blocking factors and feeding regimen covariate(s). h. **Intercurrent event(s)**: Including but not limited to treatment discontinuation. Details will be provided in the SAP.
To assess the proportion of infants who achieve a specified change in WAZ from baseline to Day 56	a. **Population**: The PP population will be used for the analysis. b. **Endpoint**: Proportion of infants with a ≥ 0.3, 0.4, and 0.5 change in WAZ from baseline (Day 1) to Day 56 visit. c. **Population level summary**: Frequency and percentages of infants with a ≥ 0.3, ≥ 0.4, and ≥ 0.5 change in WAZ from baseline (Day 1) to Day 56 in the Bi-26 and placebo groups. d. **Intercurrent event(s)**: Including but not limited to treatment discontinuation. Details will be provided in the SAP.
To assess the proportion of infants who achieve a specified WAZ at Day 56	a. **Population**: The PP population will be used for the analysis. b. **Endpoint**: Proportion of infants with a WAZ >-2 at Day 56 visit. c. **Population level summary**: Frequency and percentages of infants with a WAZ >-2 at Day 56 in the Bi-26 and placebo groups. d. **Intercurrent event(s):** Including but not limited to treatment discontinuation. Details will be provided in the SAP.
To assess the re-hospitalization rate	a. **Population**: The PP population will be used for the analysis. b. **Endpoint**: Number of re-hospitalizations for acute non-surgical illness through the Day 56 visit. c. **Population level summary**: Frequency (and percentages) of re-hospitalizations in the Bi-26 group compared to placebo. d. **Intercurrent event(s)**: Including but not limited to treatment discontinuation. Details will be provided in the SAP.

9.7.1. Other Secondary Analyses

The safety of Bi-26 supplementation will be evaluated through EoS in the Safety population, which includes, but is not limited to, the frequency (and percentages) of AEs, and all SAEs through the Day 90 visit. Individual results and descriptive statistics will be provided by treatment group.

Individual results and descriptive statistics for presence of *B. infantis* in stool will be provided at each time point by treatment group.

9.7.2. Exploratory Analyses

The stool and blood biomarkers at specified timepoints will be analyzed using descriptive statistics.

Refer to Section 8.6 for biomarker testing. Details of the exploratory biomarker analyses, including potential additional exploratory engraftment analyses, will evolve from the initial test set to potential verification set. These will be included in separate operational and/or analysis plans. Results of these additional exploratory objectives may be reported separately from the main clinical study report.

10. Supporting Documentation and Operational Considerations

10.1. Regulatory, Ethical, and Study Oversight Considerations

10.1.1. Regulatory and Ethical Considerations

This study will be conducted in accordance with the protocol and with:

- consensus ethical principles derived from international guidelines including the Declaration of Helsinki and CIOMS International Ethical Guidelines
- applicable ICH GCP Guidelines
- applicable laws and regulations.

The protocol, protocol amendments, ICF, and other relevant documents (e.g., advertisements) must be submitted to an EC/IRB by the investigator and reviewed and approved by the IRB/EC before the study is initiated.

Any amendments to the protocol will require EC/IRB approval and approval by health authorities where applicable, before implementation of changes made to the study design, except for changes necessary to eliminate an immediate hazard to study participants.

The investigator will be responsible for the following:

Providing written summaries of the status of the study to the EC/IRB annually or more frequently in accordance with the requirements, policies, and procedures established by the IRB/EC

Notifying the IRB of SAEs or other significant safety findings as required by EC/IRB procedures.

Providing oversight of the conduct of the study at the site and adherence to requirements of ICH guidelines, the EC/IRB, and all other applicable local regulations.

10.1.2. Financial Disclosure

Investigators and sub-investigators will provide the sponsor with sufficient, accurate financial information as requested to allow the sponsor to submit complete and accurate financial certification or disclosure statements to the appropriate regulatory authorities. Investigators are responsible for providing information on financial interests during the course of the study and for 1 year after completion of the study.

10.1.3. Informed Consent Process

The investigator or his/her representative will explain the study to the participant's parent(s)/legal guardian and answer all questions regarding the study. The participant's parent(s)/legal guardian must be informed that their participation is voluntary.

The participant's parent(s)/legal guardian will be required to sign a statement of informed consent that meets the requirements of local regulations, ICH guidelines, and the ECs/IRBs or study centers.

Note that the participant's mother is the preferred individual to sign the informed consent given the protocol requirements for some feedings to be from breast milk and for collection of information on maternal antibiotic and probiotic usage.

However, the participant's other parent or legal guardian can provide consent if necessary. If the mother is not the consenting parent, the study requirements should be reviewed with the participant's mother to ensure understanding of study activities and ability/willingness to comply. The participant's mother will also sign the ICF for collection of her medical information described in the protocol.

The medical record must include a statement that written informed consent was obtained before the participant was screened and enrolled in the study and the date the written consent was obtained. The authorized person obtaining the informed consent must also sign the ICF.

Participant's consenting parent(s)/legal guardian must be re-consented to the most current version of the ICF during their infant's participation in the study.

A copy of the ICF must be provided to the participant's parent(s)/legal guardian prior to any study-related procedure.

Any withdrawal of consent for sample testing will be documented in the eCRF.

Refer to Section 5.3 regarding rescreening.

10.1.3.1. Informed Consent Form

Participant's consenting parent/legal guardian will be told that they are free to refuse to have their infant participate and may withdraw their consent at any time and for any reason during the study period. Any test results from the samples collected before withdrawing consent may still be used for the study.

The informed consent form will be obtained using a written ICF approved by the IRB/EC and signed and dated by the participant's consenting parent/legal guardian.

The ICF will contain a separate section that addresses the use of remaining mandatory samples for optional exploratory biomarker research. The investigator or authorized designee will explain the objectives of the exploratory research to the participant's consenting parent/legal guardian.

Participant's consenting parent/legal guardian must agree to the exploratory research; otherwise the participant cannot be in the study.

10.1.4. Data Protection

Participants will be assigned a unique identifier by the sponsor. Any participant records or datasets that are transferred to the sponsor will contain the identifier only; participant names or any information which would make the participant identifiable will not be transferred. The participant's consenting parent must be informed that the infant's medical records may be examined by Clinical Quality Assurance auditors or other authorized personnel appointed by the sponsor, by appropriate EC/IRB members, and by inspectors from regulatory authorities.

The participant's mother, and the other consenting parent or legal guardian if not the mother, must be informed that the mother's and infant's personal study-related data will be used by the sponsor in accordance with local data protection law. The level of disclosure must also be explained.

10.1.5. Dissemination of Clinical Study Data

10.1.6. Data Quality Assurance

All participant data relating to the study will be recorded by an eCRF using an electronic data capture (EDC) system transmitted to the sponsor or designee electronically (e.g., laboratory data). The investigator is responsible for verifying that data entries are accurate and correct by electronically signing the eCRF.

The investigator must maintain accurate documentation (source data) that supports the information entered in the eCRF.

The study will be monitored regularly by the sponsor or its designee throughout the study period.

The investigator must permit study-related monitoring, audits, IRB/EC review, and regulatory agency inspections and provide direct access to source data documents.

Monitoring details describing strategy (e.g., risk-based initiatives in operations and quality such as Risk Management and Mitigation Strategies and Analytical Risk-Based Monitoring), methods, responsibilities and requirements, including handling of noncompliance issues and monitoring techniques (central, remote, or on-site monitoring) are provided in the Monitoring Plan.

The sponsor or designee is responsible for the data management of this study including quality checking of the data.

The sponsor assumes accountability for actions delegated to other individuals (e.g., CRO).

Study monitors will perform ongoing source data verification to confirm that data entered into the eCRF by authorized site personnel are accurate, complete, and verifiable from source documents; that the safety and rights of participants are being protected; and that the study is being conducted in accordance with the currently approved protocol and any other study agreements, ICH GCP, and all applicable regulatory requirements.

10.1.7. Record Retention

Records and documents pertaining to the conduct of this study must be retained by the investigator for a minimum of 10 years after study completion unless local regulations or institutional policies require a longer retention period.

No records may be destroyed during the retention period without the written approval of the sponsor. No records may be transferred to another location or party without written notification to the sponsor.

10.1.8. Source Documents

Source documentation consists of existing medical records and/or study records developed and maintained by the investigator. Source documents provide evidence for the existence of the participant and substantiate the integrity of the data collected and are to be filed at the investigator's site. Data recorded on source documents will be transcribed onto eCRFs using an EDC system.

Data entered in the eCRF that are transcribed from source documents must be consistent with the source documents or the discrepancies must be explained. The investigator may need to request previous medical records or transfer records, depending on the study.

For the purpose of monitoring and auditing the study, source documentation will consist of existing medical records and/or study records developed and maintained by the investigator.

10.1.9. Study and Site Closure

The sponsor designee reserves the right to close the study site or terminate the study at any time for any reason at the sole discretion of the sponsor. Study sites will be closed upon study completion. A study site is considered closed when all required documents and study supplies have been collected and a study site closure visit has been performed.

The investigator may initiate study site closure at any time, provided there is reasonable cause and sufficient notice is given in advance of the intended termination. Reasons for the early closure of a study site by the sponsor or investigator may include but are not limited to:

- failure of the investigator to comply with the protocol, the requirements of the EC/IRB or local health authorities, the sponsor's procedures, or GCP guidelines
- inadequate recruitment of participants by the investigator
- discontinuation of further study intervention development.

At the discretion of the sponsor, all materials and supplies provided to the investigator will be returned or disposed of in compliance with local regulatory requirements upon authorization from the sponsor, upon study completion. The investigator or designated clinical site staff will notify the EC/IRB when the study has been completed.

10.1.10. Publication Policy

The results of this study may be published or presented at scientific meetings. If this is foreseen, the investigator agrees to submit all manuscripts or abstracts to the sponsor before submission. This allows the sponsor to protect proprietary information and to provide comments.

The sponsor will comply with the requirements for publication of study results. In accordance with standard editorial and ethical practice, the sponsor will generally support publication of multicenter studies only in their entirety and not as individual site data. In this case, a coordinating investigator will be designated by mutual agreement.

Authorship will be determined by mutual agreement and in line with International Committee of Medical Journal Editors authorship requirements.

10.1.11. Financing and Insurance

Financing and insurance information is provided as a separate agreement.

10.2. Adverse Events and Serious Adverse Events: Definitions and Procedures for Recording, Evaluating, Follow-up, and Reporting

10.2.1. Definition of AE

AE Definition
An AE is any untoward medical occurrence in a patient or clinical study participant, temporally associated with the use of study intervention, whether or not considered related to the study intervention. NOTE: An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease (new or exacerbated) temporally associated with the use of study intervention.
Events Meeting the AE Definition
Any abnormal laboratory test results (hematology, clinical chemistry, or urinalysis) or other safety assessments (e.g., ECG, radiological scans, vital signs measurements), including those that worsen from baseline, considered clinically significant in the medical and scientific judgment of the investigator (i.e., not related to progression of underlying disease). Exacerbation of a chronic or intermittent pre-existing condition including either an increase in frequency and/or intensity of the condition. New conditions detected or diagnosed after study intervention administration even though it may have been present before the start of the study. Signs, symptoms, or the clinical sequelae of a suspected drug-drug interaction. Signs, symptoms, or the clinical sequelae of a suspected overdose of either study intervention or a concomitant medication. Overdose per se will not be reported as an AE/SAE unless it is

an intentional overdose taken with possible suicidal/self-harming intent. Such overdoses should be reported regardless of sequelae.
Events NOT Meeting the AE Definition
Any clinically significant abnormal laboratory findings or other abnormal safety assessments which are associated with the underlying disease, unless judged by the investigator to be more severe than expected for the participant's condition. The disease/disorder being studied or expected progression, signs, or symptoms of the disease/disorder being studied, unless more severe than expected for the participant's condition. Medical or surgical procedure (e.g., endoscopy, appendectomy): the condition that leads to the procedure is the AE. Situations in which an untoward medical occurrence did not occur (social and/or convenience admission to a hospital). Anticipated day-to-day fluctuations of pre-existing disease(s) or condition(s) present or detected at the start of the study that do not worsen.

10.2.2. Definition of SAE

If an event is not an AE per definition above, then it cannot be an SAE even if serious conditions are met.

An SAE is defined as any untoward medical occurrence that, at any dose:
i. Results in death
j. Is life-threatening The term 'life-threatening' in the definition of 'serious' refers to an event in which the participant was at risk of death at the time of the event. It does not refer to an event, which hypothetically might have caused death, if it were more severe.
k. Requires inpatient hospitalization or prolongation of existing hospitalization In general, hospitalization signifies that the participant has been detained (usually involving at least an overnight stay) at the hospital or emergency ward for observation and/or treatment that would not have been appropriate in the physician's office or outpatient setting. Complications that occur during hospitalization are not necessarily SAEs. If a complication prolongs hospitalization or fulfills any other serious criteria, the event is serious. When in doubt as to whether "hospitalization" occurred or was necessary, it should be considered as an SAE. Hospitalization for elective treatment of a pre-existing condition that did not worsen from baseline is not considered an adverse event.
l. Results in persistent disability/incapacity

- The term disability means a substantial disruption of a person's ability to conduct normal life functions.
- This definition is not intended to include experiences of relatively minor medical significance such as uncomplicated headache, nausea, vomiting, diarrhea, influenza, and accidental trauma (e.g., sprained ankle) which may interfere with or prevent everyday life functions but do not constitute a substantial disruption.

m. Is a congenital anomaly/birth defect

n. Other situations:

- Medical or scientific judgment should be exercised in deciding whether SAE reporting is appropriate in other situations such as important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the participant or may require medical or surgical intervention to prevent one of the other outcomes listed in the above definition. These events should usually be considered serious.

  Examples of such events include invasive or malignant cancers, intensive treatment in an emergency room or at home for allergic bronchospasm, blood dyscrasias or convulsions that do not result in hospitalization, or development of drug dependency or drug abuse.

10.2.3. Recording and Following Up SAEs

SAE Recording

- Care will be taken not to introduce bias when detecting SAEs. Open-ended and non-leading verbal questioning of the participant is the preferred method to inquire about occurrences.
- When an SAE occurs, it is the responsibility of the investigator to review all documentation (eg, hospital progress notes, laboratory reports, and diagnostics reports) related to the event.
- The investigator will then record all relevant SAE information in the eCRF.
- After the initial SAE report, the investigator is required to proactively follow each participant at subsequent visits/contacts. All SAEs will be followed until resolution, stabilization, the event is otherwise explained, or the participant is lost to follow-up.
- It is **not** acceptable for the investigator to send photocopies of the participant's medical records to the sponsor in lieu of completion of the SAE eCRF page.
- There may be instances when copies of medical records for certain cases are requested by the Medical Monitor, the IDMC or the sponsor. In this case, all participant identifiers, with the exception of the participant number, will be redacted on the copies of the medical records before submission.

- The investigator will attempt to establish a diagnosis of the event based on signs, symptoms, and/or other clinical information. Whenever possible, the diagnosis (not the individual signs/symptoms) will be documented as the SAE.
- SAEs will be assessed for intensity and causal relationship to the study intervention.

SAE Follow-up and Resolution

The investigator is obligated to perform or arrange for the conduct of supplemental measurements and/or evaluations as medically indicated to elucidate the nature and/or causality of the SAE as fully as possible. This may include additional laboratory tests or investigations, histopathological examinations, or consultation with other health care professionals.

If a participant dies during participation in the study or during a recognized follow-up period, the investigator will provide the sponsor with a copy of any post-mortem findings including histopathology, if available.

New or updated information will be recorded in the originally completed eCRF.

The investigator will submit any updated SAE data to the sponsor within 24 hours of receipt of the information.

The onset and resolution dates of the event and medical care taken in response to the event will be documented.

SAEs will be considered resolved when the condition returns to normal or returns to the participant's baseline status as established on Study Day 1, or when the condition has stabilized with the expectation that it will remain chronic.

If the event has not resolved by the final study visit, it will be documented as "ongoing" on the eCRF, however, follow-up of the SAE must continue until resolved or the condition has stabilized. Information recorded on the eCRF must be substantiated in the source documents.

The resolution date to be recorded on the eCRF is the last date on which the participant experienced the SAE.

Assessment of Intensity

The investigator will make an assessment of intensity for each SAE reported during the study and assign it to 1 of the following categories:

- Grade 1, Mild: An event that is easily tolerated by the participant, causing minimal discomfort and not interfering with everyday activities.
- Grade 2, Moderate: An event that causes sufficient discomfort and interferes with normal everyday activities.
- Grade 3, Severe: An event that prevents normal everyday activities. An AE that is assessed as severe should not be confused with a SAE. Severe is a category utilized for rating the intensity of an event; and both AEs and SAEs can be assessed as severe.
- Grade 4 Potentially life-threatening symptoms, causing inability to perform basic self-care functions with intervention indicated to prevent permanent impairment, persistent disability, or death.

  An event is defined as 'serious' when it meets at least 1 of the predefined outcomes as described in the definition of an SAE, NOT when it is rated as severe.

Assessment of Causality

The investigator is obligated to assess the relationship between study intervention and each occurrence of each SAE. The investigator will assess whether the SAE is either:

Related: An SAE is considered related to study intervention if there is a reasonable possibility that the study intervention contributed to the SAE.
or
Not related: There is no reasonable possibility that the SAE is causally related to administration of the study intervention. There are other more likely causes for the SAE.

The investigator will use clinical judgment to determine the relationship.

Alternative causes, such as underlying disease(s), concomitant therapy, and other risk factors, as well as the temporal relationship of the event to study intervention administration will be considered and investigated.

For each SAE, the investigator **must** document in the medical notes that he/she has reviewed the SAE and has provided an assessment of causality. There may be situations in which an SAE has occurred and the investigator has minimal information to assess causality to include in the initial report. However, **it is very important that the investigator always provide an assessment of causality with the initial submission of the SAE data.**

The investigator may change his/her assessment of causality when considering additional follow-up information and submit an SAE follow-up report with the updated causality assessment. The causality assessment is one of the criteria used when determining regulatory reporting requirements.

Assessment of Outcome
The outcome of each SAE must be reported to the sponsor. For analysis purposes, the outcome for serious adverse events will be determined on the final study visit. Outcome of all SAEs will be classified as one of the following: •Resolved •Resolved with sequelae •Ongoing •Death.

10.2.4. Reporting SAEs

SAE Reporting to CRO via an Electronic Data Collection Tool
• The primary mechanism for reporting an SAE to the CRO will be the electronic data collection tool. • If the electronic system is unavailable, then the site will use the paper SAE data collection tool (see next section) in order to report the event within 24 hours. • The site will enter the SAE data into the electronic system as soon as it becomes available. • After the study is completed at a given site, the electronic data collection tool will be taken off-line to prevent the entry of new data or changes to existing data. • If a site receives a report of a new SAE from a study participant or receives updated data on a previously reported SAE after the electronic data collection tool has been taken off-line, then the site can report this information on a paper SAE form (see next section) or to the Medical Monitor by telephone. • Contacts for SAE reporting can be found in the Study Manual.
SAE Reporting to CRO via Paper Case Report Form
• Email transmission of the SAE paper CRF is the preferred method to transmit this information. • In rare circumstances and in the absence of email, notification by telephone is acceptable with a copy of the SAE data collection tool sent by overnight mail or courier service. • Initial notification via telephone does not replace the need for the investigator to complete and sign the SAE CRF pages within the designated reporting time frames. • Contacts for SAE reporting can be found in the Study Manual.

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12. Protocol Amendments

Overall Rationale for changes in Version 3.0:

The primary purpose of this protocol update was to combine the enrollment activities with the Day 1 (randomization) visit and clarify other study procedures. The major changes incorporated into this protocol (Version 3.0) relative to the prior approved version (Version 2.0) are summarized below. Editorial and formatting changes are not included in this summary.

Section # and Name	Description of Change	Brief Rationale
Page 3	Added Principal Investigator signatory page.	To have signature recorded together with other study documents in a single location
Throughout the document	Removed the word "unsolicited" before 'AEs'.	This study does not include solicited AEs, so all AEs are unsolicited. However, to avoid any confusion, the word "unsolicited" was removed to clarify that all AEs will be recorded.
Synopsis, Objectives and Endpoints (Table 1) and Section 9.7 Table 5 Secondary Estimands	Updated text in Table 1 and Table 5 to align wording across the tables.	For clarification
Synopsis, Schedule of Activities (SOA Table 2) and Main Study Schema (Figure 1), and Section 4.1	Combined enrollment activities with the Day 1 (randomization) visit. Added text to indicate that screening may occur on Day 1 with enrollment and randomization, or separately within the protocol-specified window.	To clarify the timing of screening, enrollment, and randomization activities
Synopsis, SoA (Table 2)	Updated procedural details to align throughout the protocol. Updates include separating medication collection for the infant and the infant's mother, specifying time points for provision and collection of diaries, and addition of explanatory footnotes.	For clarification
Section 4.4 End of Study Definition	Clarified the definition of study completion for an individual participant.	For clarification

Section # and Name	Description of Change	Brief Rationale
Section 5.3 Screen Failures and Section 10.1.3 Informed Consent Process	Replaced text that stated 'rescreening will not be allowed', to allow for rescreening if screening visit is outside of the 7-day planned window, due to a delay in hospital discharge.	For flexibility during the screening process
Section 5.1 Inclusion criteria	Specified that enrollment is on Day 1.	To clarify when the participant is enrolled in the study
Section 5.1and 5.2, Inclusion/exclusion criteria, Section 6 Study intervention and throughout document	Replaced "mother" with "participant's parent/legal guardian" unless only the mother can perform a particular task.	To allow more flexibility at participant's home so that another parent or legal guardian may perform some study activities
Section 6.2Measures to Minimize Bias: Randomization and Blinding	Clarified individuals who will be blinded and unblinded.	For clarification
Section 6.3 Study Intervention Compliance	Removed confirmation of refrigeration temperature using a temperature logger.	Temperature data loggers will not be used to monitor the study intervention.
Section 6.4 Feeding history	Added details to be recorded, including whether the full dose was taken, and whether a repeat dose was administered.	For clarification
Section 7.1 Discontinuation	Added details regarding investigator decision to stop the study intervention for an individual participant.	For clarification
Section 7.3 Replacement of Participants	Added new section "Replacement of Participants" and a statement that participants will not be replaced.	To specify that participants will not be replaced if early discontinuation from study occurs
Section 8.1 Demographic and Baseline Characteristics	Reworded text and added details regarding what will be collected at screening and at Day 1.	For clarification
Section 8.3.3 Vital Signs	Replaced respiratory rate with pulse oximetry	For clarification

Section # and Name	Description of Change	Brief Rationale
Section 8.4.5 Independent Data Monitoring Committee (IDMC)	Added further details about possible IDMC recommendations and about sponsor decision to terminate the study for safety reasons.	For clarification
Section 10.1.11 Financial Disclosure	Added this new section.	For clarification
Section 10.1.3 Informed Consent Process	Included a statement that the participant's mother will also sign the Informed Consent Form to allow collection of her medical information.	For clarification
Protocol Version History and Summary of Changes page 4, and Section 12 Protocol Amendments	Moved text from page 3 to newly created Section 12, Protocol Amendments.	To move details from previous amendment changes to end of document and provide new amendment changes at the beginning of the document

Minor editorial changes were made throughout the protocol for corrections or to clarify text.

Overall Rationale for changes in Version 2.0:

The protocol was amended to include a sub-study to monitor the temperatures of the refrigerators used in the main study. The aim of this optional, prospective, observational sub-study is to collect information on reliability of refrigeration in the geographic areas where the study is being conducted and where *B. infantis* supplementation may be recommended by WHO, if supported by clinical data. As of 2020, 733 million people did not have access to electricity, and over 75% of people living in sub-Saharan Africa did not have access. In order to get a geographical cross-representation in this sub-study, each clinical site will be asked to enroll up to 20% of their overall study population into the sub-study.

Section # and Name	Description of Change	Brief Rationale
1.1 Synopsis 4.4 Optional Sub-Study and Table 2 Schedule of Activities.	This section was added to the protocol to specify use of a temperature data logger over a 2-week period in the home refrigerators of a subset of the study participants.	To collect information on reliability of refrigeration in the geographic areas where the study is being conducted.
Minor changes were made throughout the protocol including the following:		
Throughout protocol	The term 'formula' was replaced with 'water' for product reconstitution.	To specify that water can be used to mix with the Bi-26 or placebo, instead of formula, if breastmilk is not available for a given dose.
Section 2.1 Background	Text added to clarify GRAS status information.	To provide additional information on the GRAS designation
Section 2.2. Risk Benefit Assessment	New data from the SYNERGIE study added.	To provide evidence to support the potential for positive clinical outcomes with *B. infantis* supplementation.
[redacted]		
Section 8.6 Biomarkers	Blood volume changed from 2 mL to 4mL.	To allow for adequate blood volume for testing.

Minor editorial changes were made throughout the protocol for corrections or to clarify text.

Signature Page for: Gates MRI-MNK01-301 Protocol Version 4.0 (12 Sep 2022)

Gateway RIM Document #: MNK01-CLIN-000003 v4.0

Document Approvals	
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eSignature Approval	[redacted] Biometrics & Data Management 14-Sep-2022 13:15:44 GMT+0000
eSignature Approval	[redacted] Chief Medical Officer 14-Sep-2022 13:37:30 GMT+0000