Statistical Analysis Plan: Gates MRI-MNK01-301

Study 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
Study Number:	Gates MRI-MNK01-301
Study Phase:	Phase 3
Sponsor:	Bill and Melinda Gates Medical Research Institute
NCT #:	
Version:	Version 1
Date:	14 September 2023

Confidentiality Statement

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2 DOCUMENT HISTORY

Document	Date
Original SAP version 1	18 August 2023

3 SIGNATURE PAGE

Study 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

Study Number: Gates MRI-MNK01-301

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4 LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

ACR	Assessment/collection/result
ATC	Anatomical Therapeutic Chemical
AE	Adverse event
Bi-26	Strain of *Bifidobacterium longum, B. infantis*
CFB	Change from baseline
CHAIN	The Childhood Acute Illness and Nutrition Network
CI	Confidence interval
CRF	Case report form
CRP	Complete Randomization Probability
DACP	Dynamic Allocation with Complete Randomization
DHS	Demographic and Health Survey
eCRF	Electronic case report form
EC	Ethics committee
EDC	Electronic data capture
EoS	End of study
GA	Gestational age
HIV	Human immunodeficiency virus
ICF	Informed consent form
IDMC	Independent Data Monitoring Committee
IFDC	Infant feeding diary card
ITT	Intent-to-treat
MedDRA	Medical Dictionary for Regulatory Activities
mITT	Modified Intention-to-treat
MMRM	Mixed model for repeated measures
MUAC	Mid-upper arm circumference
PCI	Potentially clinically important
PE	Physical examinations
PP	Per Protocol
PT	Preferred term
RTSM	Randomization and Trial Supply Management

SAE	Serious adverse event
SAP	Statistical analysis plan
SAS®	Statistical Analysis Software
SD	Standard deviation
SES	Socioeconomic status
SoA	Schedule of Activities
SOC	System organ class
TE	Temperature excursion
TEAE	Treatment emergent adverse event
TLF	Table, listing and figure
WAZ	Weight-for-age Z-score
WHO	World Health Organization
WLZ	Weight-for-length Z-score

5 INTRODUCTION

The purpose of this SAP is to describe the framework for the reporting, summarization, and statistical analysis methodology of the safety and efficacy parameters measured throughout the study. It is based on Protocol Gates MRI-MNK01-301 dated 12 September 2022 (Version 4).

6 STUDY OBJECTIVES AND ENDPOINTS

6.1 Primary Objective

Table 1. Primary objective

Objective	Estimand
To evaluate the change in weight (standardized for age) of infants receiving Bi-26	a) **Population**: The Modified Intent to Treat (mITT) population will be used for the analysis. b) **Endpoint**: Weight-for-age z-score (WAZ) change from baseline (CFB) (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.

6.2 Secondary Objectives

Table 2. Secondary Objectives

Objective	Estimand
Key Secondary	
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.
Secondary	
To estimate the treatment response over time associated with Bi-26	a) **Population**: The mITT population will be used for the analysis. b) **Endpoint**: WAZ change from baseline (Day 1) over time through the Day 90 visit (longitudinal assessment) by duration of dosing. c) **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). d) **Intercurrent event(s):** Including but not limited to treatment discontinuation.
To assess the proportion of infants who achieve a specified change in WAZ from baseline to Day 56	a) **Population**: The Safety population will be used for the analysis. b) **Endpoint**: Proportion of infants with a $\geq$ 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 $\geq$ 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.

To assess the proportion of infants who achieve a specified WAZ at Day 56	a) **Population**: The Safety 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 the placebo groups. d) **Intercurrent event(s)**: Including but not limited to treatment discontinuation.
To assess the re-hospitalization rate	a) **Population**: The Safety 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 and the placebo groups. d) **Intercurrent event(s)**: Including but not limited to treatment discontinuation.

6.2.1 Secondary Objectives

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.

6.2.2 Exploratory Objectives

The stool and blood biomarkers at specified timepoints will be analyzed using descriptive statistics. Details of any additional exploratory biomarker analyses, including potential engraftment analyses, will evolve from the initial test set to verification set. These will be included in separate operational and/or analysis plans. Results of these additional exploratory objectives will be reported separately from the main clinical study report.

7 STUDY DESIGN CONSIDERATIONS

7.1 Study Design

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 (30 to 120 days) 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 approximately 90 days.

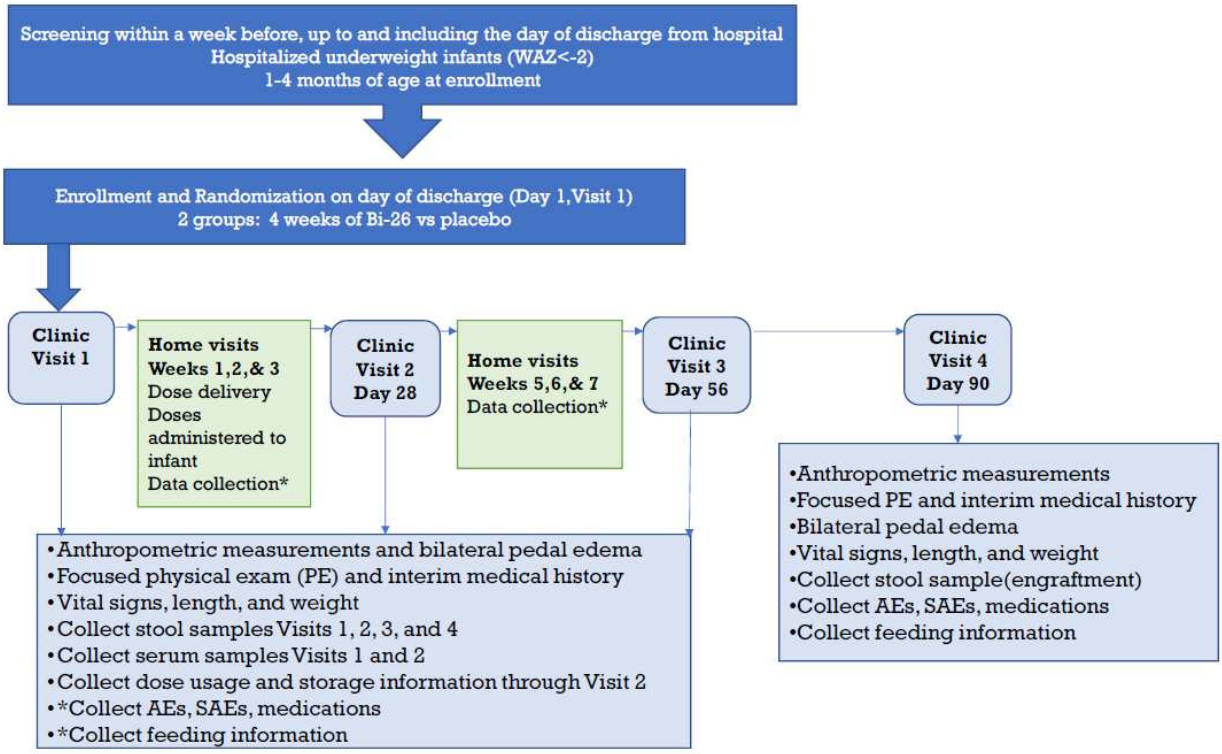
Screening will take place during the infant's hospitalization, within a week of anticipated discharge. A participant discharged to a nutrition rehabilitation unit would still be considered a hospital discharge. Enrollment and randomization will take place on the day of hospital discharge (Day 1) and after the infant participant has completed the acute stabilization phase of treatment, including fluid rehydration and antibiotic course as needed. 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.

All participants will be given either Bi-26 supplementation, or product matched placebo (lacking the active ingredient), based on randomization. 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 (eg, 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 health center.

7.1.1 Study Schema

Figure 1. Study schema



Schedule of Activities is presented in Appendix 1.

7.1.2 Independent Data Monitoring Committee

While there is no formal interim analysis, an IDMC will review unblinded safety data during closed sessions and make recommendations to the sponsor. Separate TLFs will be developed for use in IDMC sessions according to the IDMC charter. A watermark will be used to distinguish between open and closed session TLFs.

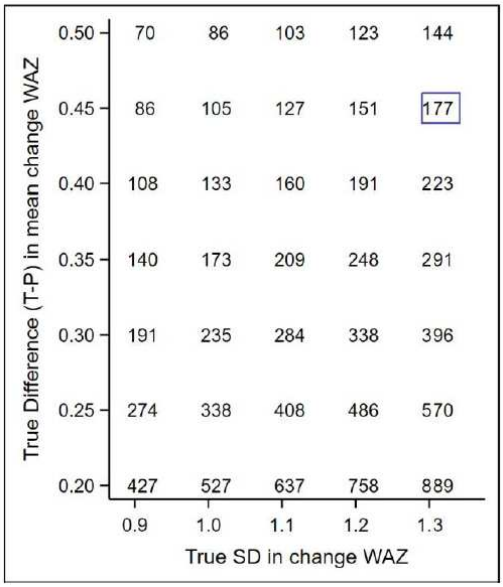
7.1.3 Justification of Sample Size

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 in 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 are 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%

7.2 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 (Bangladesh, Kenya, Pakistan, Tanzania)
2. Age at enrollment (Study Day 1):30 to 59 days of age, 60 to 120 days of age
3. Discharge destination (home, nutritional rehabilitation unit)
4. WAZ (≤ -3 versus > -3)

Dynamic randomization will be utilized to balance the treatment groups across the study population, site level, stratum level, and within stratification factors. Target randomization is approximately 2/3 of participants with WAZ between -2 and -3 and approximately 1/3 with WAZ ≤ -3. The distribution of participants in WAZ strata will be monitored throughout the study in order to determine if a stratum needs to be temporarily/permanently closed for enrollment.

See Sections 11.5.1 and 11.6.6 for the calculations of age and WAZ to be used for stratification, respectively.

7.2.1 Details of dynamic randomization

Treatment allocation will be done dynamically as participants are randomized. WAZ will be calculated in the Rave EDC system using sex, weight and age and the appropriate look-up table with a custom-built function. Rave RTSM will use the WAZ score calculated in the Rave EDC system for the stratification according to WAZ. Overall treatment balance of 1:1 between Bi-26 and Placebo will be achieved using dynamic randomization.

Dynamic randomization in Rave makes use of a covariate-adjusted dynamic allocation method [2, 4, 5, 6]. It is similar to other dynamic randomization methods by making use of an imbalance measure to assess how far off the allocations are from the targeted allocation between treatment arms, using weights to assign priority to stratification and study factors for treatment balance, and using the preceding allocations to assign the next treatment allocation.

In order to do the treatment allocation, the DACP algorithm in the Medidata Rave RTSM system will be used. The parameter CRP is a mandatory input which determines the probability of using either random allocation of treatments or using the minimization algorithm to determine the next treatment allocation. A random integer, say r_1 , is generated between 0 and 100. If

- $\frac{r_1}{100} \leq CRP$ then the system picks the treatment allocation randomly between Bi-26 or Placebo with a probability of 50%
- $\frac{r_1}{100} > CRP$ then the system uses the minimization algorithm to assign treatment allocation based on imbalance scores.

A CRP of 15% is chosen to use the minimization algorithm in most of the treatment allocations. This will result in roughly 85% of treatment allocations to be assigned using the minimization algorithm. The final choice of the CRP was determined through simulations run in Medidata Rave RTSM, comparing different scenarios with different distributions of WAZ strata, different CRP values, as well as input from Medidata experts.

The weights used to assign the priority of balance to each factor is given by an integer value from 0 to 5 for each of the stratification variables (in this case WAZ, Age, Country, and Discharge destination) as well as for study (overall), stratum and site balance. The same weight is assigned to all levels of the stratification factors. The weights chosen for each of the relevant factors are presented in Table 3. The final choice of weights was also based on the simulations and expert input described above. High priority in balance is given to Study and WAZ as we aim for 1:1 ratio of treatments overall, and within WAZ strata.

The minimization algorithm determines the imbalance scores of assigning the next participant to be randomized to Bi-26 or Placebo. The treatment allocation least likely to cause imbalance based on these scores will be assigned.

The following formula is used to calculate the weighted imbalance score for treatment i:

$$Im\_score_i = \sum_j w_j Im_{ij}$$

where w_j is the weight associated with factor j and Im_{ij} is the relative imbalance due to assigning the next participant to be randomized to treatment i. The calculations for the different treatments are done separately, assuming the new participant is randomized to the respective treatment. Given that the target allocation between treatment groups is 1:1, that *n* participants have been randomized for factor *j* (in the level of the new participant), that *m* out of *n* participants have been assigned to treatment *i*, the relative imbalance for factor *j* and treatment *i*, Im_{ij}, is calculated as

$$Im_{ij} = 2 \times \left| 0.5 - \frac{m+1}{n+1} \right|$$

where

$$m\text{: \# } participants\ already\ allocated\ to\ treatment\ i\ in\ factor\ j\ (relevant\ level)$$

$$n\text{: \# } participants\ already\ randomized\ in\ factor\ j\ (relevant\ level)$$

Note that Im_{ij} is calculated assuming the $n + 1^{th}$ participant is randomized to treatment *i*.

Table 3. Weights Assigned to Factors Used in Dynamic Allocation

Factor	Weight
Study	3
Strata	1
Site	1
WAZ	3
Age	2
Country	1
Discharge destination	1

The final algorithm for treatment allocation of a participant is the following:

1. Determine the imbalance within factor, Im_{ij}, of assigning the next participant to treatment i, for each factor, given treatment allocations within each factor up to that point
2. Draw a random integer, say r_1, between 0 and 100
3. If:
   a. $\frac{r_1}{100} \leq CRP$: assign next treatment allocation using a random draw (complete randomization).
   b. $\frac{r_1}{100} > \text{CRP}$: assign next treatment allocation using minimization.
4. Assign treatment allocation
   a. If a random draw is used:
      i. Draw random integer between 0 and 100, r_2.
      ii. If $\frac{r_2}{100} \leq 0.5$ then allocate to Placebo.

iii. If $\frac{r_2}{100} > 0.5$ then allocate to Bi-26.

b. If minimization is used:

i. Use calculated imbalances scores, Im_score_{Pl} and Im_score_{bi26} for placebo and Bi-26 scores respectively.

ii. If $Im\_score_{Pl} < Im\_score_{bi26}$ assign the participant to Placebo.

iii. If $Im\_score_{Pl} > Im\_score_{bi26}$ assign the participant to Bi-26.

iv. If $Im\_score_{Pl} = Im\_score_{bi26}$ then a treatment is allocated randomly, as with complete randomization (see step 4(a) above).

7.3 Efficacy Measures

The following endpoints will be analyzed for primary efficacy analysis:

- Change in WAZ from baseline to Day 56 visit in the Bi-26 group.

For the secondary endpoints the following will be analyzed:

- Change in weight (in grams) from baseline to Day 56 visit in the Bi-26 group.
- Change in WAZ over time from baseline to Day 90 visit (longitudinal assessment) by duration of dosing.
- Proportion of infants with a ≥ 0.3, ≥ 0.4, and ≥ 0.5 change in WAZ from baseline (Day 1) to Day 56 visit.
- Proportion of infants with WAZ > 2 at Day 56 visit.
- Proportion of re-hospitalizations for acute non-surgical illness through Day 56 visit.
- Presence of *B. infantis* in engraftment stool samples on Days 1, 28, 56, and 90.

7.4 Safety Measures

The safety endpoints to be analyzed include:

- SAEs from Day 1 through the Day 90 visit (EoS)
- AEs from Day 1 through Day 90 visit (EoS)

7.5 Exploratory Measures

The following biomarker data will be collected to be used for exploratory samples:

- Fecal pH and Bristol or similar stool scale based on stool samples on Days 1, 28, and 56.
- Inflammatory biomarkers and host/or bacterial metabolic analysis on Days 1 and 28.

8 STUDY POPULATIONS

8.1 Analysis Populations

Agreement and authorization of participants included/excluded from the PP population will be obtained prior to database lock and unblinding. A summary table containing the number of participants in each of the populations defined below along with any reasons for exclusions will be provided.

8.1.1 All Enrolled Participants

All participants randomly assigned to study intervention.

8.1.2 Intention to Treat Population

All participants randomly assigned to study intervention. A participant will be programmatically included in the ITT population if the participant has a randomization number and date. Participants will be analyzed according to the intervention they were randomized to.

8.1.3 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.

8.1.4 Modified Intention to Treat 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 to.

8.1.5 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. Important protocol deviations will be evaluated individually by the sponsor to decide whether it has basis to exclude a participant from the PP population. Deviation from study intervention compliance (see Section 11.4) is defined as compliance of less than 80%. Participants will be analyzed according to the intervention they actually received (for details refer to Section 11.1).

8.2 Subgroups

To assess consistency of effects, subgroup analyses may be performed in selected subgroups such as:

- WAZ strata: < -3 and between -3 and -2 (boundaries excluded).
- Age: 30 to 59 days, 60 to 120 days.

- Country: Bangladesh, Kenya, Pakistan, Tanzania.
- Discharge destination: Home, Nutritional Rehabilitation Unit.
- All participants who received 100% of doses during treatment.
- Method of delivery: C-section, vaginal birth.
- Bilateral pedal edema at baseline: Yes, No.
- Prior Infant Antibiotic use: Yes, No.
- Concomitant Infant Antibiotic/Probiotic use through Day 56 visit: Yes, No.
- Proportion of feedings through Day 56 from breastmilk: 100%, 75 to 99%, 50 to 74%, 25 to 49%, < 25%.

Other subgroups may be considered in the final analysis. Summary statistics for subgroups will be reported for subgroups with greater than 5 participants in each of the treatment arms. Further details on subgroup analysis are provided in the relevant efficacy and safety sections below.

9 CHANGES IN CONDUCT OR PLANNED ANALYSES FROM THE PROTOCOL

- Dynamic randomization will include all stratification variables, including WAZ in determining the treatment allocation.
- Populations for secondary endpoints previously PP, changed to Safety with results to be reported for PP populations as sensitivity analysis.

10 OVERALL STATISTICAL CONSIDERATIONS

10.1 General Conventions

Descriptive statistics will be presented for continuous data with applicable decimal precision as follows in relation to the source data (indicated as N+x), with a maximum of 3 decimals to be displayed:

- Number (N).
- Mean, (N+1).
- Number of missing observations, (N).
- Standard deviation (SD), (N+1).
- Median, (N+1).
- Minimum, (N+1).
- Maximum, (N+1).

Absolute and relative frequencies will be used to describe categorical variables. Unless otherwise specified, the denominators for percentages will be the number of participants in each intervention group with non-missing data for the variable of interest at the summarized visit. Percentages will be presented with 1 decimal place and a percent sign (%).

All statistical testing will be 2-sided and performed at the 0.05 level. When reporting the results of significance tests p-values will be reported. All confidence intervals will be 2-sided at the 95% level, unless otherwise stated.

Study day is calculated relative to the first study intervention administration on Day 1.

- If the current ACR date is after study intervention administration:

  Study Day = (Current ACR Date – Study Intervention administration Date) + 1

- If the current ACR date is before study intervention administration:

  Study Day = (Current ACR Date – Study Intervention administration Date)

Study day will not be calculated if either the current ACR or the study intervention administration date is incomplete or missing.

The following periods for the study are defined:

- Treatment Period: Day 1 through last dose of participant.
- Follow-up Period: Day of last dose of participant + 1 up to EoS.

SAS® code of SAS® procedures that will be used to perform efficacy and safety analyses is provided in Appendix 3.

10.2 Baseline Definition

Baseline is defined as the last available value prior to the participant receiving the first dose of the study intervention. If the potential Baseline ACR is on Day 1 (and time is not recorded), it is assumed that the ACR occurred before the first dose of the study intervention was received.

CFB is calculated as:

CFB = Observed value – Baseline Value

10.3 Handling of Missing Data

10.3.1 Missing or Partial Dates for Adverse Events

Missing or partial AE start dates will be imputed for the purpose of determining whether the AEs are treatment emergent. Data handling rules for missing or partial start/stop date for AEs are detailed in Appendix 2Table 6. The missing or partial dates will be displayed in the data listings as reported on the eCRF rather than the imputed dates.

10.3.2 Missing or Incomplete Dates for Prior or Concomitant Medications

Missing or partial medication start or stop dates will be imputed for the purpose of determining whether the medication is taken concomitantly. Data handling rules for missing or partial start/stop date medications are detailed in Appendix 2 Table 7. The missing or partial dates will be displayed in the data listings as reported on the eCRF rather than the imputed dates.

10.3.3 Missing Efficacy data

Missing efficacy data will not be imputed.

10.4 Interim Analysis

No interim analysis is planned.

10.5 Pooling Strategy for Study Sites

Data will be pooled by country: Bangladesh, Kenya, Pakistan, and Tanzania (the sites that are included in each geography should be identified clearly). Analysis of the primary endpoint will include country in the model as a covariate. A random effect for site may be included based on model comparisons with and without the effect.

10.6 Visit Windows/Unscheduled Visits

Data at each visit are to be captured within the following windows for each of the intended visits:

Table 4. Visit windows

Visit name	Visit window
Screening	≤ 7 days before randomization
Randomization	Day of hospital discharge, Day 1
Clinic Visit 1	Day 1
Home Visits at Week 1	Within ± 1 day of Day 7, ie, Days 6-8
Home Visit at Week 2	Within ± 1 day of Day 14, ie, Days 13-15
Home Visit at Week 3	Within ± 1 day of Day 21, ie, Days 20-22
Clinic Visit 2	Within ± 1 day of Day 28, ie, Days 27-29
Home Visit at Week 5	Within ± 2 days of Day 35, ie, Days 33-37
Home Visit at Week 6	Within ± 2 days of Day 42, ie, Days 40-44
Home Visit at Week 7	Within ± 2 days of Day 49, ie, Days 47-51
Clinic Visit 3	Within ± 2 days of Day 56, ie, Days 54-58
Clinic Visit 4	Within ± 2 days of Day 90, ie, Days 88-92

Additional visit windowing will not be applied. Unscheduled visits will not be included in by-visit summary tables or analysis but may contribute to the Baseline value and listed in by-participant data listings. In the case of a retest (same visit number assigned), the latest available test result as provided in the data transfer for that visit/time point will be used for by-visit summaries.

11 STATISTICAL ANALYSIS METHODS

11.1 Participant Disposition

The following will be presented in a summary table by treatment and overall, for disposition:

- Number of participants screened.
- Number of screen failures.
- Reasons for screen failures.
- Number of participants re-screened.
- Number of participants randomized (ITT population).
- Number of participants randomized but not treated.
- Number of participants who received treatment (Safety population).
- Number of participants who completed the 28-day study intervention period.
- Number of participants who discontinued treatment with reasons.
- Number of participants who completed study up to Day 56 visit.
- Number of participants who withdrew from study prior to Day 56 visit with reasons.
- Number of participants who completed study (up to Day 90 visit).
- Number of participants who withdrew from the study between Days 56 and 90 visits with reasons.

The denominators for the number of participants who completed the 28-day study intervention period and discontinued treatment with reasons will be the obtained from the Safety population. A participant listing for disposition for all participants will be presented including treatment arm, country, study site, date of first and last dose, completed/discontinued treatment with reason and date, completed/withdrew from study with reason and date.

A table summarizing the populations for analysis (including the reasons for exclusion) will be presented by treatment group and overall. A listing will be presented for the ITT population with population excluded from and reasons for exclusion from analysis population for mITT and PP.

A participant listing with randomization information for the ITT population will be provided, including WAZ and age at enrollment (Study Day 1), country, discharge destination date and time of randomization and treatment group. For all participants who are screen failures the following information will be listed: informed consent date, reason for screen failure and eligibility criteria not met.

Important protocol deviations are a subset of protocol deviations that may significantly impact the completeness, accuracy and/or reliability of study data or that may significantly affect a participant's rights, safety or well-being. Important protocol deviations will be summarized in a table by treatment group and overall and all protocol deviations will be listed, including date of deviation, deviation category (important/unimportant), standard protocol deviation term and protocol deviation description.

11.2 Demographics and Baseline Characteristics

Demographics and baseline characteristics will be summarized using descriptive statistics by treatment group and overall. The denominators for percentages will be the number of participants in each treatment group with non-missing data for the variable of interest for the ITT population. The number of participants with missing data at baseline, without percentages, will be displayed for each of the variables. Two participant listings will be presented with treatment group, one for demographic variables and the other for baseline variables as listed below, for the ITT population.

The following demographic and baseline variables will be summarized and listed:

- Country (Bangladesh, Kenya, Pakistan, Tanzania).
- Age (days) at enrollment – both numeric and categorical (30-59 days, 60-120 days).
- Sex (Male, Female).
- Race (Asian, Black, Indian, White, Other).
- Ethnicity (Bantu, Bengali, Cushite, Kalenjin, Kikuyu, Luo, Luyha, Nilo-Hamite, Pashtun, Punjabi, Sairiki, San, Sindhi, Other).
- Infant been exposed to HIV (Human immunodeficiency virus) (Yes, No).
- Type of HIV exposure (Hospital, Household, Infected Mother, Other, Unknown).
- Infant HIV status, only positive will be indicated as it will be recorded in the medical history.
- Weight (grams).
- Length (cm).
- Mid-upper arm circumference (MUAC).
- Head circumference (cm).
- Skinfold thickness at the subscapular and triceps (mm).
- Bilateral pedal edema (Yes, No).
- Weight-for-age z-score (WAZ) – both numeric and categorical (-3 to -2, ≤ -3).
- Weight-for-length z-score (WLZ).
- Birth weight (grams).
- Gestational age (weeks).
- Birth delivery method (C-Section, Vaginal birth).
- Discharge destination (Home, Nutritional Rehabilitation Unit).
- Primary type of feeding the infant has received since birth (Exclusively breastmilk, Formula exclusively, A mix of breastmilk and other types of feedings, Other milk exclusively).
- Antibiotics received within prior 30 days (Yes, No).
- Have refrigerator in house (Yes, No).

See Section 11.5.1 for the calculation of WAZ, Section 11.6.6 for the calculation of WLZ and the derivations of baseline values of MUAC and head circumference.

11.2.1 Vaccinations

The number and percentage of participants who received vaccinations up to the time of screening will be presented by treatment and overall, in a table for each vaccination recorded. Concomitant vaccinations will be summarized similarly in a separate table. A participant listing including the date and name of vaccination will be provided. The tables and listing will be presented for the ITT population.

11.2.2 Socioeconomic status Estimation

An estimation of SES will be captured using a survey to assess household wealth which is publicly available from CHAIN and based on the DHS 7 questionnaire [1].

Maternal education and type of water use will be presented in a summary table using frequencies and proportions for the ITT population. The categories are given by:

- Highest level of maternal education (None, Primary, Secondary, Above Secondary, Unknown, Other, N/A care home).
- Type of water use (Treated water, Untreated water).

Type of water use is derived from "Do you usually do anything to the water to make it safer to drink?" as follows:

- Treated water: Bleach/chlorine, strain through a cloth, let it stand and settle, Use water filter, solar disinfection, boil, Other.
- Untreated water: None.

Highest level of maternal education, if anything is done to water to make it safer to drink and type of water use will be presented in a participant listing.

11.2.3 Medical History

A full medical history 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.

Medical history terms will be coded using the latest version of the MedDRA.

Medical history, including prior procedures, will be summarized by treatment group and overall, in a table by SOC and, PT for the Safety population. The number and percentage of participants who had at least 1 condition will be shown. Participants with multiple medical histories within a SOC or PT are counted only once for that SOC and PT. A participant listing for the ITT population will be presented.

No imputation of partial or missing dates will be performed for medical history and study days will not be presented for these cases.

11.3 Prior and Concomitant Medications and Procedures

A prior medication is defined as having started and ended prior to first dose of study intervention. A concomitant medication is defined as either having started prior to first dose of study intervention and ended on/after first dose of study intervention, is ongoing at Baseline or, having started on/after first dose of study intervention. Medications will be coded by site of action and therapeutic and clinical characteristics using WHO's ATC Classification.

The number and percentage of participants reporting prior and concomitant medications will be summarized separately by ATC Class and preferred name, by treatment group and overall.

The same table will be presented for concomitant medications for the mothers of the participants.

The following ATC class/terms for probiotics and antibiotics will be used to identify the participants for the subgroup analysis based on infant concomitant antibiotic/probiotic use (see Section 8.2):

- A07FA01 Lactic acid producing organisms (probiotics).
- A07FA02 Saccharomyces boulardii (probiotics).
- A07FA51 Lactic acid producing organisms, combinations (probiotics).
- J01 (antibiotics).
- J04 (antibiotics).

Prior/concomitant status (according to the definition provided at the start of this section) will be derived using the medication start and stop dates. For partial dates, see imputation rules in Appendix 2.

Reason for use, dates of administration including start and end dates, dosage information including dose and frequency of any medication or vaccine (including over-the-counter or prescription medicines, vitamins, and/or herbal supplements) that the participant was receiving at the time of screening or received during the study will be presented in participant listings for infants and mothers.

All procedures will be classified according to the latest version of MedDRA. The number and percentage of participants reporting concomitant procedures will be summarized separately by SOC and PT, by treatment. Concomitant procedures are defined as having been performed on or after the date of the first dose of study intervention. Procedures will be presented in a participant listing. The listing will include the start and stop dates of the procedures, whether it is related to an AE, reason for procedure, interpretation of procedure and findings.

The Safety population will be used to present prior and concomitant medication and procedures tables. The ITT population will be used for listings.

11.4 Study Intervention Compliance and Exposure

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 administered and why. Deviations from the planned dosing regimen will be recorded in the eCRF.

The compliance is defined as

$$CS = 100 \times \frac{Number\ of\ doses\ administered}{Number\ of\ days\ on\ study\ intervention}\%$$

where

- Number of doses administered: is the actual number of doses administered as per exposure eCRF. If a dose was re-administered due to vomiting within 30 minutes/spitting out the dose will be counted as one dose. Deviations from planned dosing regimen will be used to consider if doses were used but not administered for any reason. According to the CRF completion guidelines, doses that were spilled without any part administered, were recorded as zero doses used. If a second dose was used and administered on the same day, it was recorded in a second record in the database.
- Number of days on study intervention: calculated as the number of days during the treatment period which participant could have received treatment. Days on which treatment/study was discontinued does not form part of denominator.

Descriptive statistics of the compliance will be summarized in a table by treatment group and visit (Day 7, 14, 21, 28, Combined Day 1 to 28). For weekly summaries, compliance is calculated based on the period between the preceding visit and the current. The frequency and percentage of participants who achieved 100% compliance score over the entire treatment period will be presented by treatment group in the same table.

The number of doses administered will be presented in a separate table using descriptive statistics (mean, SD, median, min, max) by treatment group and visit. Additionally, the number and percentage of participants will be presented in the same table according to the following intervals for doses administered:

- 28 doses (100% of doses taken)
- 14 to 28
- < 14

The proportion of infants who had x% of doses for each of the dose administer and reconstitution scenarios (see Section 11.4.2), including partial doses, doses mixed in breastmilk, doses mixed in water and doses vomited within 30 minutes of administration, will be summarized using summary statistics in a table by treatment according to the following categories:

- 100%
- 75% to 99%

- 50% to 74%
- 25% to 49%
- < 25%

A histogram of compliance scores will be presented by treatment group for the compliance scores across the treatment period (Day 1 to 28). An additional histogram by treatment group will be presented for number of doses administered.

The exposure listing will include date of study intervention administration, whether the dose is the first dose or the last, deviations from planned dosing regimen, reason for non-compliance, what study powder was mixed in, vomiting after intake of study intervention and whether or not a second dose was administered following vomiting.

Another listing will be provided with compliance scores across weekly intervals during the treatment period, as well as over the entire treatment period. It includes the start date of the interval for which compliance is calculated, number of days on treatment in interval, number of doses of study intervention used, number of doses vomited within 30 minutes, number of doses administered and the calculated compliance.

The Safety population will be used for the table summaries. The summary of study intervention compliance table will also be presented for the mITT population. Listings will be presented for the mITT population.

11.4.1 Dose storage

The following data will also be collected at each visit during the treatment phase (see Appendix 1) for study intervention storage information:

- where the study intervention is being stored (at the local health centre, the home of a participant in a refrigerator, the home of a participant in a cooler, or in a refrigerator at a neighbour's home). The final categories may be adapted to appropriately reflect the data collected.
- if refrigeration was off during storage (and how many doses were compromised).

Place of storage will be summarized using frequencies and percentages by treatment in a table for entire treatment period. A listing will be presented including place of storage, if refrigeration was off during storage, how long refrigeration was off, and number of doses compromised. This analysis will be presented for the Safety population.

11.4.2 Infant Feeding Diary Card

The IFDC captures information about the feeding the infant received on a particular study day through the Day 90 visit, which includes the following for each day:

- Number of breastmilk feedings
- Number of formula feedings
- Number of solid food feedings

- Number of other feedings
- Total number of feedings

as well as info on whether or not the infant got all of the mixed study powder in, how the study powder was re-constituted and if vomiting occurred after administration of study intervention.

For each participant the proportion of feedings from breastmilk through the Day 56 Visit will be calculated as follows:

$$Prop\ breastmilk\ feedings = \frac{Sum\ of\ \#\ BM\ feedings\ per\ day}{Sum\ of\ \#\ Total\ feedings\ per\ day}$$

for available data up to Day 56 visit. Days with missing values in feeding information will not contribute to the above calculation.

The proportion of infants who had x% of feedings from breastmilk through the Day 56 visit will be summarized using summary statistics in a table by treatment according to the following categories:

- 100%
- 75% to 99%
- 50% to 74%
- 25% to 49%
- < 25%

A diary card entry on any given day is non-compliant, if no values were recorded for the feeding information and the total number of feedings for the day was missing. The proportion of infants who had x% of compliant entries will be presented by treatment group according to the following categories:

- 100%
- 75% to 99%
- 50% to 74%
- 25% to 49%
- < 25%.

Infant diary card results will be listed for the safety population. The listing will include whether diary card was returned, date of distribution, date of receipt, number of feedings for each type and the total.

11.5 Efficacy

11.5.1 Calculation of Weight-for-age z-score

WAZ is calculated at Screening and all clinical visits (Days 1, 28, 56, and 90).

The WAZ score is calculated in EDC using the approach defined by the WHO [2]. Given the weight (kg), sex and age (in days) of a participant, the WAZ is calculated as

$$WAZ = \frac{\left(\left(\frac{Weight}{M}\right)^{L} - 1\right)}{L \times S}$$

where L, M, and S are pre-derived parameter estimates generated by the WHO. L, M, and S are conditional on the Sex and Age (days) of the infant. The lookup tables for the values of L, M, and S can be found on the WHO website [2]. Distinct tables for males and females are given.

WAZ is calculated directly in EDC and need not be derived. WAZ is rounded off to 2 decimals. The value for WAZ (at enrollment) after rounding off is used for randomization and defining the strata (-3 < WAZ < -2, WAZ ≤ -3) (also used for study eligibility).

11.5.2 Primary outcome: CFB in WAZ at Day 56

11.5.2.1 Analysis of endpoint

WAZ will be summarized using descriptive statistics for actual and CFB values by visit and treatment group.

A MMRM will be used to estimate the difference in mean CFB between Bi-26 and Placebo, adjusting for covariates baseline WAZ, age (in days), country and discharge destination. The model will use CFB in WAZ as outcome and include the following effects:

- Fixed effects: Treatment, Visit (categorical), Treatment*Visit interaction, WAZ at baseline (continuous), Age (in days) at baseline, Country, Discharge destination
- Random intercepts: participant.

and will be fit using data from all planned follow-up visits where WAZ was measured, ie, Day 28, 56, and 90 visits. The adjusted interaction effects (Treatment*Visit) will be presented as estimates of the difference in CFB between treatment groups at each follow-up visit (Day 28, 56, and 90). The associated 95% CIs and p-values will be presented. PROC MIXED will be used to fit the models. SAS code for the models is provided in Appendix 3. If the WAZ score is not normally distributed, an appropriate transformation, such as the natural log, will be used as outcome for analysis. Normality will be investigated using q-q plots.

A participant listing of WAZ will be presented with actual values and CFB.

The descriptive table, model and listing will use the mITT population. The summary table will be repeated for the PP population.

Line graphs with the means and 95% CIs for WAZ will be presented by treatment group for visits Baseline, 28, 56, and 90.

11.5.2.2 Subgroup analysis

Summary statistics may be presented by treatment and visit for subgroups as specified in Section 8.2 for WAZ. The mean difference in CFB with 90% confidence intervals between treatment and placebo will be presented at each visit.

A forest plot will be presented for selected subgroups showing the 90% CIs for mean CFB in WAZ at Day 56 visit by treatment group.

The mITT will be used for this analysis.

11.5.2.3 Association between study intervention compliance and CFB in WAZ

In order to evaluate the relationship between CFB in WAZ at Day 56 and study intervention compliance, the Pearson's and Spearman's correlations between CFB in WAZ at Day 56 and study intervention compliance will be presented by treatment group in a table. A scatter plot by treatment group will be presented with CFB in WAZ at Day 56 as the dependent variable and study intervention compliance as the independent variable. PROC CORR will be used to produce the correlations.

The same scatter plot with CFB in weight (grams) will also be presented. The mITT will be used for this analysis.

11.5.2.4 Sensitivity Analyses

11.5.2.4.1 Per Protocol population

As a sensitivity analysis, the same model presented in Section 11.5.2.1 will be presented for the PP population with corresponding results.

11.5.2.4.2 Adjustment for possible confounding variables

In order to quantify the impact of confounding factors, the same model presented in Section 11.5.2.1 will be adjusted for possible confounding factors that may include:

- study intervention compliance (see Section 11.4)
- proportion of breast milk feedings (see Section 11.4.2)
- infant concomitant antibiotic or probiotic use through the Day 56 visit (yes/no)
- primary place of storage (see Section 11.4.1).

For each of the confounders to be included in the analysis, the same model presented in Section 11.5.2.1 will be run adjusted by the confounder. If the p-value of the overall test for the confounder < 0.2 it will be included in the final model. The same estimates will be presented as for the primary efficacy analysis in a summary table.

11.5.2.4.3 WAZ adjusted for gestational age

For a sensitivity analysis WAZ is adjusted for gestational age by using the following age calculation:

$$Age_{GA}(weeks) = Age\ (weeks) - (37 - GA(weeks))$$

where:

- Age (weeks) is calculated as age (days)/7 and rounded down to closest integer,
- GA(weeks) is reported gestational age in weeks.

WAZ_{GA} is re-calculated using Age_{GA} (days) which is calculated as Age_{GA}(weeks) multiplied by 7 and rounded down to closes day.

WAZ_{GA} will be summarized similarly to WAZ by treatment and visit. A listing will be included with actual and CFB values for WAZ_{GA}. Summaries for WAZ_{GA} will not be presented for any subgroup.

Finally, the same model used in Section 11.5.2.1 is run using CFB in WAZ_{GA} at Day 56 visit as outcome, and baseline WAZ_{GA} and Age_{GA} (days) as covariates in the model instead of WAZ and Age (days). The same summary table will be presented for WAZ_{GA} for the mITT population.

Missing gestational age will not be imputed.

11.5.3 Key Secondary Outcome: CFB in Weight (grams) at Day 56

11.5.3.1 Analysis of endpoint

Weight (grams) will be summarized using descriptive statistics for actual and CFB values by visit and treatment group. CFB values will be presented in the summary table in gram units. To obtain CFB in grams, weight (kg) as captured in the eCRF will be multiplied by 1,000.

The same model presented in Section 11.5.2.1 will be used to estimate the difference in mean CFB (in grams) between Bi-26 and Placebo at Day 56, adjusting for baseline weight (grams), age, country, and discharge destination. The adjusted interaction effects (Treatment*Visit) will be presented as estimates of the difference in CFB between treatment groups at each follow-up visit (Day 28, 56, and 90). The associated 95% CIs and p-values will be presented. PROC MIXED will be used for the analysis. Normality will be investigated using q-q plots. If weight is not normally distributed an appropriate transformation, such as the natural log, will be used as outcome for analysis.

A participant listing of weight will be presented with actual values (in kilograms and grams) and CFB (in grams).

The descriptive table, model and listing will be presented for the mITT population. The summary table will be repeated for the PP population.

Line graphs with the means and 95% CIs for weight (grams) will be presented by treatment group for visits Baseline, 28, 56, and 90.

11.5.3.2 Subgroup analysis

Summary statistics, including 90% confidence intervals for difference in CFB will be presented by treatment and visit for subgroups as specified in Section 8.2 for weight. The mITT population will be used for this analysis.

11.5.4 Secondary outcome: Change over time in WAZ by duration of dosing

11.5.4.1 Analysis of endpoint

The difference in the change over time will be represented as the estimated difference in CFB at Day 90 using the results generated by the model as described in Section 11.5.2.1 for the PP population.

In order to take duration of dosing into account, the model described above will be adjusted by study intervention compliance (see Section 11.4) and presented in separate tables for the mITT and PP populations.

11.5.5 Secondary outcome: Proportion of infants with increase in WAZ ≥ 0.3, 0.4 and 0.5 at Day 56

11.5.5.1 Analysis of endpoint

The proportion of infants with increase in WAZ ≥ 0.3, 0.4, and 0.5 at Day 56 with 95% CIs will be estimated and presented in a table by treatment group. The binomial mid-P method will be used to estimate the CIs.

Log-binomial regression will be used to estimate the risk ratio of rates of increase in WAZ > 0.3, 0.4 and 0.5 at Day 56 between Bi-26 and Placebo, adjusting for the following covariates: baseline WAZ, age (in days), country and discharge destination. The adjusted risk ratio (exponentiated coefficient) with 95% CI will also be presented in the same summary table as the proportion estimates. If the overall rate is less than 15% of all participants or the log-binomial model does not converge, then logistic regression will be used in the place of log-binomial regression and odds ratios will be reported instead of risk ratios, which will be a reasonable estimate of the risk ratios. Separate models will be fitted for each of the outcomes of increase in WAZ > 0.3, 0.4 and 0.5. PROC GLIMMIX will be used for this analysis.

The Safety and PP populations will be used for this analysis.

Increases will be indicated as flags in the WAZ listing described in Section 11.5.2.1.

11.5.6 Secondary outcome: Proportion of infants with WAZ > -2 at Day 56

11.5.6.1 Analysis of endpoint

The proportion of infants with WAZ > -2 at Day 56 with 95% CIs will be estimated and presented in a table by treatment group. The binomial mid-P method will be used to estimate the CIs.

Log-binomial regression will be used to estimate the risk ratio of WAZ > -2 rates at Day 56 between Bi-26 and Placebo, adjusting for the following covariates: baseline WAZ, age (in days), country and discharge destination. The adjusted risk ratio (exponentiated coefficient) with 95% CI will also be presented in the same summary table as the proportion estimates. If the overall rate is less than 15% of all participants or the log-binomial model does not converge, then logistic regression will be used in the place of log-binomial regression and odds ratios will be reported instead of risk ratios, which will be a reasonable estimate of the risk ratios. PROC GLIMMIX will be used for this analysis.

The Safety and PP populations will be used for this analysis.

Increases will be indicated as flags in the WAZ listing described in Section 11.5.2.1.

11.5.7 Re-hospitalization rates through Day 56

The number of re-hospitalizations for acute non-surgical illness by Day 56 per participant will be summarized using descriptive statistics and will be presented in a table by treatment group.

Negative-binomial regression will be used to estimate the incidence rate ratio of re--hospitalization at Day 56 between Bi-26 and Placebo, adjusting for the following covariates: baseline WAZ, age (in days), country and discharge destination. The adjusted rate ratio (exponentiated coefficient) with 95% CI will also be presented in the same summary table as the descriptive statistics.

The number and proportion of infants that were re-hospitalized for acute non-surgical illness by Day 56 with 95% CIs will be presented in a table by treatment group. The binomial mid-P method will be used to estimate the CIs.

Log-binomial regression will be used to estimate the risk ratio of re-hospitalization rates at Day 56 between Bi-26 and Placebo, adjusting for covariates baseline WAZ, age (in days), country and discharge destination. The adjusted risk ratio (exponentiated coefficient) with 95% CI will also be presented in the same summary table. If the overall re-hospitalization rate is less than 15% of all participants or the log-binomial model does not converge, then logistic regression will be used in the place of log-binomial regression. Odds ratios will be reported instead of risk ratios, which will be a reasonable estimate of the risk ratios.

PROC GLIMMIX will be used for this analysis.

Re-hospitalizations for acute non-surgical illness will be listed in a separate listing for all participants, including date/time of admission, associated SAE, preferred term of SAE and date/time of discharge if discharged.

The Safety and PP populations will be used for this analysis.

11.5.7.1 Subgroup analysis

The number and proportion with 90% CIs of infants that were re-hospitalized for acute non-surgical illness by Day 56 may be presented in a table by treatment group for subgroups as specified in Section 8.2. The binomial mid-P method will be used to estimate the CIs. Results will be presented for the mITT population.

11.5.8 Presence of *B. infantis* in stool

The proportion of infants with presence of *B. infantis* with 95% CIs will be estimated and presented in a table by treatment group for Days 1, 28, 56, and 90. The binomial mid-P method will be used to estimate the CIs. The definition of "presence of *B. infantis"* will be refined following qPCR validation activities in which a threshold for presence/absence will be determined.

A listing will be provided including date of sample collection, Bristol stool scale, fecal pH, and presence of *B. infantis*.

The mITT and PP populations will be used for this analysis.

11.5.9 Exploratory outcomes

Exploratory stool will be collected across enrollment, Day 28 and Day 56 visits and serum samples will be collected across enrollment and Day 28 visits. Further details on analysis of these outcomes will be discussed in an exploratory analysis plan.

11.5.10 Interim Analysis

No formal interim analysis is planned for this study.

11.6 Safety Analyses

All safety analyses will be performed for the Safety population.

11.6.1 Adverse Events

Safety assessments include SAEs and AEs from the time the ICF is signed through Day 90 (or at the date of last visit/study discontinuation).

An AE is considered treatment emergent if it started on or after the date of first dose of study intervention administration.

An AE overview table containing the number and percentage (expressed in percent) of participants with AEs from Day 1 through Day 90 will be summarized by treatment group for the following:

- Any AE
- Any TEAE
- TEAEs related to study intervention
- TEAEs by maximum severity (Mild, Moderate and Severe)
- TEAEs leading to study intervention discontinuation
- TEAEs leading to study withdrawal
- Serious TEAEs
- Serious TEAEs with Outcome of Death

Additionally, the number and percentage of participants with AEs will be provided by SOC and PT and by treatment group for the following:

- Any TEAE
- TEAEs by maximum severity
- TEAEs related to study intervention
- Serious TEAEs
- Serious TEAEs related to study intervention

By-period summaries will be presented for TEAE and serious TEAEs by treatment group, where period is defined as in Section 10.1.

The overall AE summary table, AE table by SOC and PT and SAE table by SOC and PT will be presented by subgroups as specified in Section 8.2.

All AEs and SAEs will be classified according to the latest version of MedDRA.

SOC and PT will be sorted alphabetically by SOC and by decreasing frequency of preferred term in the all Bi-26 group (then alphabetically for ties). If a participant has more than one AE at a given level (eg, SOC and preferred term), the participant will only be counted once within that level. All summary tables will include the number of AEs. Missing severities and relationship will not be regarded as ‘worst case’.

The following listings will be presented:

- All AEs through to Day 90
- SAEs through to Day 90

The AE listing by participant will display all reported AEs up to Day 90 and will include the verbatim term in addition to the SOC and preferred term. This listing will also include all relevant data associated with the event: eg, date of onset, date resolved, date of first dose, date of last dose, severity, whether AE is serious, outcome, relationship to study intervention, action taken with study intervention, and required therapy. When a date is presented, the study day

associated with the date will also be displayed. The same listing will be presented for SAEs but will also include seriousness criteria.

11.6.2 Deaths

The death participant listing will include date and time of death, whether the condition under study contributed to death, primary cause of death and autopsy findings (if applicable).

11.6.3 Clinical Laboratory

No laboratory safety data are collected and therefore will not be presented for this study.

11.6.4 Electrocardiograms

No ECG data are collected and therefore will not be presented for this study.

11.6.5 Vital Signs

Vital signs, length (cm) and weight (kg) will be assessed and recorded at all clinic visits (Days 1, 28, 56, and 90). Vital sign measurements will include pulse rate (bpm), pulse oximetry (SpO2), and body temperature (ºC). 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. Length will be measured twice and both values will be recorded. The average value of the 2 recorded lengths will be used for any analysis.

A summary table with descriptive statistics for actual and CFB values, will be presented by visit and treatment group. PCI criteria for pulse rate, pulse oximetry (SpO2), and body temperature are given inTable 5.

Table 5. Vital Signs PCI

Vital Signs Parameter	PCI Criteria
Body temperature	If temperature location is Axillary: < 36.5°C or > 37.5°C
	If temperature location is Ear: < 35.8°C or > 38.0°C
	If temperature location is Forehead: < 36.4°C or > 38.0°C
	If temperature location is Rectal: < 36.6°C or > 38.0°C
Pulse Rate	< 85 bpm or > 190 bpm
SpO2	< 95%

Temperature values should be rounded off to 1 decimal before applying PCI.

The number and percentage of participants meeting PCI for each vital sign (temperature, pulse rate, and pulse oximetry) at Day 28 visit and for any post-baseline visit will be summarized by treatment group in a table.

A participant listing of vital signs will be presented with actual values and a flag for values meeting PCI criteria.

11.6.6 Anthropometrics

Anthropometrics will be assessed and recorded at all clinic visits (Days 1, 28, 56, and 90). Anthropometric measurements will include MUAC (cm), WAZ, WLZ, head circumference (cm), and skinfold thickness (mm). Details on WAZ can be found in Section 11.5.1. Head circumference and MUAC will be measured twice. The average of these measurements will be used for analysis. Skinfold thickness is measured at two locations: the subscapular and triceps. Both measurements will be presented in the outputs.

The WLZ score is calculated similarly to WAZ. Given the weight (kg), sex and length (cm) of a participant, WLZ is calculated as

$$WLZ = \frac{\left(\left(\frac{Weight(kg)}{M}\right)^{L} - 1\right)}{L \times S}$$

Where L, M, and S are pre-determined parameters describe above. The lookup tables for the values of L, M, and S can be found on the WHO website [2]. The average length of the available values from the two independent measurements will be used to derive WLZ at a specific visit. The minimum length of an infant for which a WLZ score can be calculated is 45 cm.

For lengths ≥ 45 cm, WLZ will be derived using the above formula and the relevant lookup table. For lengths < 45 cm, parameter estimates for L, M, and S are not available and therefore WLZ will be assumed to be missing.

A summary table with descriptive statistics for actual and CFB values, will be presented by visit and treatment group. A listing with actual and CFB values will be presented. Line graphs with

the means and 95% CIs for WLZ will be presented by treatment group for visits Baseline, 28, 56, and 90.

11.6.7 Bilateral Pedal Edema

The number and percentage of participants with bilateral pedal edema will be presented by treatment group for baseline and any post-baseline visit.

11.6.8 Physical Examinations

Clinically significant physical examination findings will be provided in a listing.

11.7 Pharmacokinetics/Pharmacodynamics

Pharmacokinetics/pharmacodynamics is not applicable as it is a probiotic study.

11.8 Other Relevant Data Analyses/Summaries

11.8.1 Optional Sub-study: Temperature Logger Data

Temperature logger data will be captured for Days 35 through 49. Values out of range will be indicated as such in the temperature logger data. The number and percentage of participants, with out of normal range temperature readings will be presented by country and treatment group in a summary table for those included in the sub-study in the ITT population. The proportion of participants with the number of days with out-of-range values will be shown for the following categories:

- 0 days
- 1 to 3 days
- 4 to 6 days
- 7 to 9 days
- 10 to 12 days
- $\geq$ 13 days.

The days when the logger is in transit, ie, the first and last days of temperature logger data, will be excluded from the calculation of the number of days with out-of-range values.

It is expected that 20% of ITT population will be enrolled into the sub-study. A listing will be presented for participants who were included in the sub-study in the ITT population, including daily temperature reading, time of reading and whether the reading was out of normal range.

12 REFERENCES

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13 APPENDICES

Appendix 1. Schedule of Assessments and Procedures

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 4 D 35**	**Wk 5 D 42**	**Wk 6 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
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 Protocol Section 8.3.1 full medical history and full PE.

[g] Refer to Protocol 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 Protocol 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.

Appendix 2. Imputation of Partial Dates

Table 6. Imputation Rules for Partial Dates – Adverse Events

Parameter	Missing	Additional Condition	Imputation
Start Date	D only	M and Y are prior to first study intervention dose	First day of indicated month
		M and Y is same as first study intervention dose	Date of first study intervention dose
		M and Y are after first study intervention dose	First day of indicated month
	M and D	Y is prior to first study intervention dose	01 Jan of indicated year
		Y is same as first study intervention dose	Date of first study intervention dose
		Y is after first study intervention dose	01 Jan of indicated year
	M, D, and Y	-	assumed to be TEAE
End Date	D only	M and Y are prior to last study intervention dose	Last day of indicated month
		M and Y is same as last study intervention dose	Date of last observation
		M and Y are after last study intervention dose	First day of indicated month
	M and D	Y is prior to last study intervention dose	31 Dec of indicated year
		Y is same as last study intervention dose	Date of last observation
		Y is after last study intervention dose	01 Jan of indicated year
	M, D, and Y	-	TEAE is ongoing
	-	Estimated end date is before a complete or imputed AE start date	Last day of the month of AE start date

D = day; M = month; Y = year; TEAE = treatment-emergent adverse event
Note: The imputation of end date must be later than start date

Table 7. Imputation Rules for Partial Dates – Prior and Concomitant Medications

Parameter	Missing	Additional Condition	Imputation
Start Date	D only	M and Y same as M and Y of first study intervention dosing	Date of first study intervention dose
		M and/or Y not the same as M and Y of first study intervention dosing	First day of indicated month
	M and D	Y same as Y of first study intervention dosing	Date of first study intervention dose
		Y not the same as Y of first study intervention dosing	01 January of indicated year
	M, D, and Y	none – date completely missing	Date of first study intervention dose
End Date	D only	M and Y same as M and Y of last study intervention dosing	Date of last study intervention dose
		M and/or Y not the same as M and Y of last study intervention dosing	Last day of indicated month
	M and D	Y same as Y of last study intervention dosing	Date of last study intervention dose

	Y not the same as Y of last study intervention dosing	31 December of indicated year
M, D, and Y	none – date completely missing	Date of last study intervention dose

D = day; M = month; Y = year

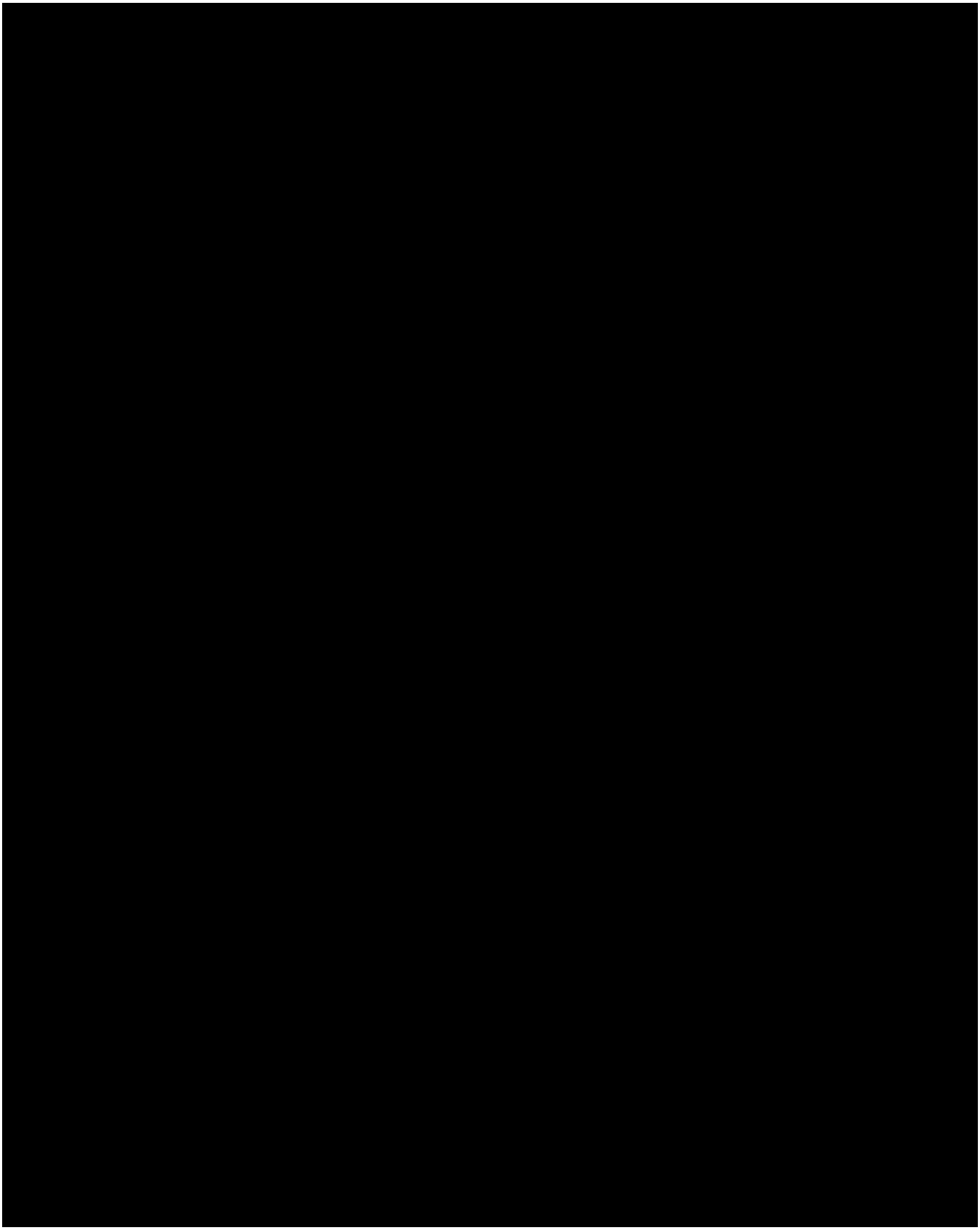
Appendix 3. Summary of Efficacy Analyses

Table 8. Analyses of Efficacy Parameters

Parameter	Population	Statistical Method	Missing Data	Interpretation
CFB in WAZ	mITT	MMRM	Observed cases	Primary analysis
CFB in WAZ	PP	MMRM	Observed cases	Sensitivity
CFB in WAZ	mITT, PP	MMRM adjusted for study intervention compliance	Observed cases	Secondary/Sensitivity
CFB in WAZ	mITT	MMRM with adjustment of confounding variables	Observed Cases	Secondary, Sensitivity
CFB in WAZ adjusted for gestational age	mITT	MMRM	Observed Cases	Sensitivity
CFB in Weight	mITT	MMRM	Observed cases	Key Secondary
Proportion of participants with increase in WAZ $\geq$ 0.3, 0.4, 0.5 at Day 56	Safety, PP	Estimation of proportions with mid-P CIs and log-binomial regression to estimate risk ratio	Observed cases	Secondary, Sensitivity
Proportion of participants with WAZ > -2	Safety, PP	Estimation of proportions with mid-P CIs and log-binomial regression to estimate risk ratio	Observed cases	Secondary, Sensitivity
Proportion of participants that were re-hospitalized	Safety, PP	Neg-binomial model to estimate incidence rate ratio	Observed cases	Secondary, Sensitivity
Proportion of participants that were re-hospitalized	Safety, PP	Estimation of proportions with mid-P CIs and log-binomial model to estimate risk ratio	Observed cases	Secondary, Sensitivity

Proportion of participants that had samples with presence of *B. infantis*	mITT, PP	Estimation of proportions with mid-P CIs	Observed cases	Secondary, Sensitivity

[redacted]



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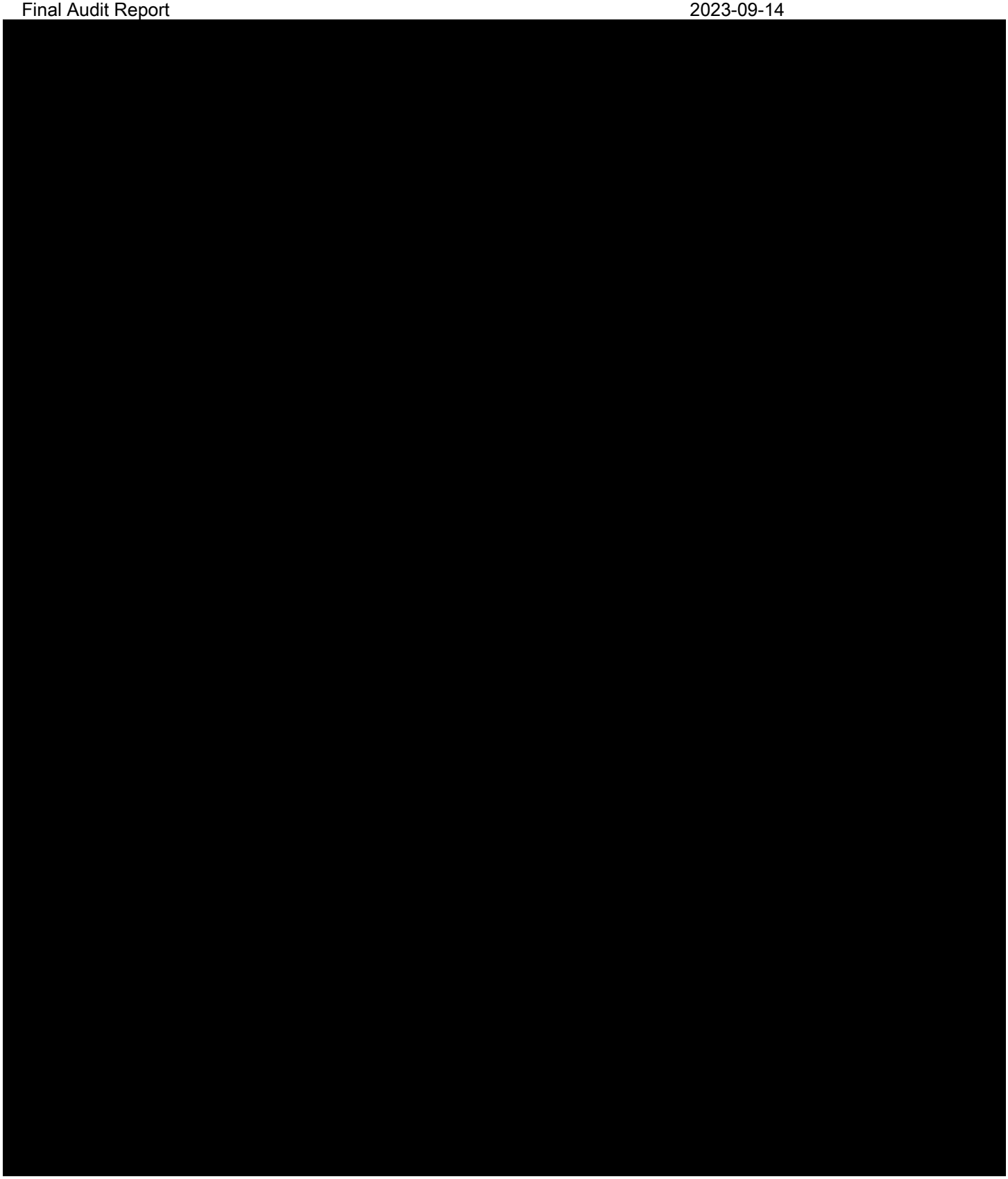

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