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Permalink

<https://escholarship.org/uc/item/95k0r2cc>

Journal

BMJ Open, 12(12)

ISSN

2044-6055

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Publication Date



2022-12-01

DOI

10.1136/bmjopen-2022-069116

Peer reviewed

BMJ Open Mitigating Infectious morbidity and Growth deficits in HIV-exposed uninfected infants with human Milk Oligosaccharide (MIGH-T MO): a randomised trial protocol

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To cite: Shivakoti R, Slogrove AL, Laughton B, *et al*. Mitigating Infectious morbidity and Growth deficits in HIV-exposed uninfected infants with human Milk Oligosaccharide (MIGH-T MO): a randomised trial protocol. *BMJ Open* 2022;**12**:e069116. doi:10.1136/bmjopen-2022-069116

► Prepublication history for this paper is available online. To view these files, please visit the journal online (<http://dx.doi.org/10.1136/bmjopen-2022-069116>).

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Received 11 October 2022
Accepted 19 December 2022



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ABSTRACT

Introduction Children who are HIV-exposed uninfected (HEU), that is, children who do not acquire HIV infection despite being born to mothers with HIV, have a higher risk of mortality, infectious morbidity and growth deficits than children who are HIV-unexposed uninfected (HUU). Prior research has focused on breast feeding and has pointed to changes in human milk oligosaccharides (HMOs) associated with maternal HIV that may influence the infant microbiome and thereby lead to these adverse outcomes. However, to our knowledge, no study has attempted to intervene along this pathway to reduce the occurrence of the adverse outcomes in children HEU. We will conduct a double-blind, randomised trial of a synbiotic intervention, which combines an HMO and probiotic, in breastfed infants HEU in South Africa to evaluate whether this intervention has promise to reduce excess infectious morbidity and growth faltering compared with controls.

Methods and analysis One hundred and forty-four breastfed infants HEU, aged 4 weeks, will be 1:1 randomised to receive either a daily synbiotic or an identical-looking placebo through age 24 weeks. Infants will be followed until age 48 weeks and outcomes of infectious morbidity, growth and biological measurements (eg, microbiota, inflammation and metabolome) will be assessed. Analyses will follow intention-to-treat principles comparing the cohorts as randomised. Infants HEU will be compared across arms with respect to the occurrence of infectious morbidity and growth outcomes through 4–24 weeks and 4–48 weeks using appropriate parametric and non-parametric statistical tests. Additionally, an observational cohort of 40 breastfed infants HUU will be recruited as a comparator group with no intervention.

Ethics and dissemination Ethical approval for this study has been obtained from the ethics committees at Columbia University and Stellenbosch University. The findings will be disseminated in publications.

Trial registration number ClinicalTrials.gov Identifier: NCT05282485. SANCTR ID number: DOH-27-122021-6543.

STRENGTHS AND LIMITATIONS OF THIS STUDY

- ⇒ A major strength of this study is the double-blind, randomised design to test the intervention.
- ⇒ There is strong observational data that support the likely efficacy of the intervention to ameliorate the infectious morbidity and growth deficits in children HIV-exposed uninfected.
- ⇒ Evaluation of biological pathways at relevant time points will provide important information on potential effects of the intervention on the infant microbiome and immune responses.
- ⇒ This study will use a proof-of-concept, futility design to evaluate whether the intervention shows 'promise' for a future larger study or alternatively a lack of promise (ie, futility).
- ⇒ Longer follow-up periods in future studies may reflect the long-term impact of the intervention on infants (if any).

INTRODUCTION

Children who are HIV-exposed uninfected (HEU) are at higher risk of mortality, infectious morbidity (respiratory infections and diarrheal disease), and poor growth outcomes compared with children who are HIV-unexposed uninfected (HUU).^{1–7} The largest increases in infectious morbidity and mortality in children HEU are observed during mid-infancy, with disparities starting in the neonatal period and persisting through infancy.^{1 7–9}

The interacting biological and social factors that account for these differences is an area of active investigation.^{10–12} Studies have focused on breast feeding and have pointed to changes in human milk oligosaccharides (HMO) associated with maternal HIV infection that may influence the infant microbiome and thereby lead to adverse outcomes.^{13–15} Among infants

HEU, higher maternal breastmilk concentration of alpha-1-2-linked fucosylated HMO (ie, 2'-fucosyllactose (2'FL) and LNFP I) and alpha-1-3/4-linked fucosylated HMOs (3-FL and LNFP II/III) were significantly associated with lower mortality during breast feeding.¹⁴ In fact, breast feeding was associated with reduced mortality in infants HEU only with higher levels of fucosylated HMOs.¹⁴ In investigations of the relationship of HMOs with infant gut microbiota, we observed differences in infant gut microbiome composition and maturity by HMO profile and there were differences by maternal HIV status.¹⁵ Taken together these studies raise the possibility that differences in HMOs between mothers with and without HIV may be one of the 'missing links' that help explain the worse outcomes observed in children HEU compared with HUU.

We propose to test an intervention that we hypothesise may ameliorate some of the adverse health outcomes observed in children HEU. We will conduct a placebo-controlled trial of supplementing breastfed infants who are HEU with a synbiotic intervention, specifically 2'FL, combined with a probiotic, specifically *Bifidobacterium longum subspecies infantis* (*B. infantis*), from 4 to 24 weeks of age to test the hypothesis that this synbiotic will have the potential to improve infectious morbidity and growth outcomes.

METHODS AND ANALYSIS

Objectives

The primary objective of this study is:

- ▶ To evaluate the effects of the synbiotic on infectious morbidity and growth while it is in place from 4 to 24 weeks of age.
- ▶ To evaluate the effects of the synbiotic on infectious morbidity and growth from 4 to 48 weeks of age.

The secondary objectives of this study are:

- ▶ To evaluate the effects of the synbiotic on biological measurements (microbiota composition and function, faecal short-chain fatty acids, plasma metabolome and markers of inflammation/growth and HMOs) while it is in place from 4 to 24 weeks of age.
- ▶ To evaluate the effects of the synbiotic on biological measurements from 24 to 48 weeks of age.

- ▶ To investigate whether the synbiotic reduces infectious morbidity and improves growth in infants who are HEU relative to HUU.
- ▶ To investigate feasibility, acceptance, tolerability and behavioural adherence with the intervention.
- ▶ To investigate whether infant gut microbiota composition, maturity and function, and markers of inflammation/growth and HMOs at baseline and over time are associated with morbidity and poor growth in children who are HEU compared with HUU.

Trial design

A two-arm, randomised, double-blind, placebo-controlled trial of 144 infants HEU (figure 1) using a futility design will evaluate the effect of the intervention. Infants HEU will be randomised 1:1 to either (A) intervention (synbiotic: 2'FL HMO+B. *infantis* probiotic) or (B) placebo (maltodextrin). Synbiotic or placebo will be administered to infants starting from 4 weeks of age and will be given daily to 24 weeks of age. Both arms will be followed to 48 weeks of age. Assessment of infant outcomes, along with other data and samples, will be collected at time points shown in table 1 below. Forty infants HUU will be followed without intervention as a control group.

Prior to the initiation of this study, we also completed a feasibility study administering the placebo of this trial, that is, maltodextrin, for 4 weeks to 10 children HEU in order to assess the acceptability, feasibility and adherence to a powder-based intervention.

Participants, interventions and outcomes

Study setting

This study will recruit 144 women who are breast feeding and living with HIV and their infants who are HEU, and additionally 40 breastfeeding women without HIV and their infants HUU. All participants will be seen at the Worcester Campus of Stellenbosch University (SU), Worcester, South Africa.

Eligibility criteria

The following criteria must be met by the mother and child for eligibility in this study:

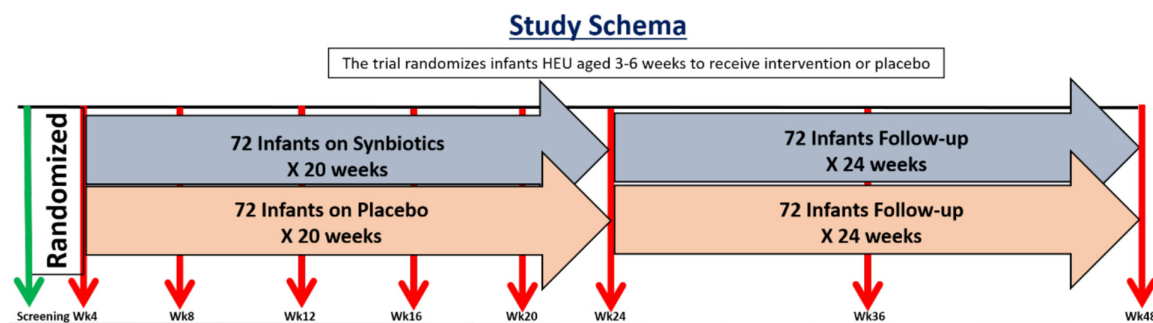


Figure 1 Study schema. Study schema showing the study design, sample size, the duration of follow-up and frequency of follow-up infants HEU. There will be another 40 infants HUU who will be followed at the same frequency without any intervention. HEU, HIV-exposed uninfected; HUU, HIV-unexposed uninfected.

Table 1 Schedule of evaluations

	Prescreening (3–7 days)	Screening (2 weeks)	Entry (4 weeks)	8 weeks	12 weeks	16 weeks	20 weeks	24 weeks	36 weeks	48 weeks
Maternal										
HIV status	X									
Informed consent for dyad		X								
BF counselling and support		X	X	X	X	X	X	X	X	X
Intervention training and support			X	X	X	X	X			
Behavioural assessment of adherence with intervention				X	X	X	X	X		
Sociodemographic data			X							
Maternal clinical and pregnancy history			X							
Maternal health status and BF practice update			X	X	X	X	X	X	X	X
Maternal blood taken and residual stored			X					X		
Soluble markers (SCFAs, metabolites and inflammatory markers)			X (10 mL)					X (10 mL)		
Viral load and CD4 count (only for HIV+)			X (10 mL)							
Breastmilk		X			X			X		
HMO measured		X			X			X		
Soluble markers (SCFAs, metabolites and inflammatory markers)		X			X			X		
Infant										
HIV status (routine care not study)	X				X (10 Wk)				X	
HIV status (study)										X
Infant clinical history			X							
Infant clinical update			X	X	X	X	X	X	X	X
Infant anthropometry			X	X	X	X	X	X	X	X
Developmental Screening Questionnaire								X		X
Infant blood taken and residual stored			X (4–6 mL)		X (4–6 mL)			X (10 mL)		X (10 mL)
HMO measured		X						X		
Metabolome		X						X		
Soluble markers (SCFAs and inflammatory markers)		X			X			X		X
Stool collection		X	X	X	X	X	X	X	X	X
Microbiome composition		X	X	X	X	X	X	X	X	X
SCFAs		X			X			X		X

Mother–infant dyads will be evaluated on the schedule shown above to 48 weeks. The schedule above is for dyads where the mother is living with HIV. Dyads where the mother does not have HIV will be on the same schedule but will omit procedures related to the intervention and maternal HIV status. Windows for each visit includes half of the time period until the next visit, after which it will be part of the next visit.

HMO, human milk oligosaccharides; SCFAs, short chain fatty acids.

Criteria for mother

1. Provision of signed and dated informed consent form.
2. Stated willingness to comply with all study procedures and availability for the duration of the study.
3. Greater than 18 years of age.
4. Currently, exclusively breast feeding and intend to breastfeed for at least another 24 weeks.
5. For Infants HEU: Mothers living with HIV documented based on medical record and with viral suppression in plasma (<400 copies/mL) documented at delivery.
6. For infants HUU: Mothers without HIV (document HIV-negative test result at delivery or screening).
7. For women with HIV: Currently on first-line standard of care antiretroviral therapy that was initiated a minimum of 12 weeks prior to delivery of the infant included in this study.
8. Has a cell phone that can be used for calls and messages.
9. Agreement to adhere to lifestyle considerations throughout the study duration.

Criteria for child

1. Up to 3–6 weeks of age.
2. Delivered from a singleton pregnancy.
3. Child is well enough to have established full breast feeding by the time of enrolment.
4. For children of mothers with HIV: At least one HIV diagnostic nucleic acid amplification test prior to enrolment which is negative and no positive test.

Exclusion criteria

A mother–child dyad who meets any of the following criteria will be excluded:

1. Severe maternal or infant illness (eg, maternal: tuberculosis, major psychiatric or neurological conditions; infant: any congenitally acquired infections, major congenital anomalies).
2. Use of immunomodulatory or immunosuppressive drugs in either mother or child prior to enrolment in the study.
3. For mothers with HIV: Mothers who are not currently receiving antiretroviral therapy or who are on regimens other than the currently recommended first-line standard of care in South Africa, that is, first line dolutegravir-based or efavirenz-based regimens.
4. Children infected with HIV.
5. Mother or infant currently taking probiotics, prebiotics or fibre supplements; or on any nutritional supplements (eg, FM85) that impact the outcomes of interest.
6. Mother or infant currently taking antibiotics for more than 14 days, excluding preventative therapies.
7. Known allergic reactions to components of the treatment or placebo.
8. Any condition that, in the opinion of the study staff, would make participation in the study unsafe, complicate interpretation of study outcome data, or otherwise interfere with achieving the aims of the study.

Note: Infant cotrimoxazole prophylaxis will be discouraged but will not be an exclusion criteria.¹⁶

Who will take informed consent?

Trained study staff at SU will obtain written informed consent, using visual consent as an aid, from mothers for themselves and their infants in-person in a private setting and in their preferred language.

Intervention

The study intervention has two components: (1) the manufactured HMO 2'FL (1.2 g) and (2) the probiotic Bi-26 *B. infantis* at a target dose of 1.5×10^9 CFU daily (~300 mg). These products are packaged together into a single-use sachet for daily use. The probiotics are freeze-dried and will only be active once they are ingested by the infants. The placebo will be 1.5 g maltodextrin daily¹⁷ packaged in an identical sachet with the same instructions. The manufacturer of both the intervention and placebo products is International Flavors and Fragrances. Mothers will be counselled on mixing the contents of the sachet with about 5 mL of expressed breastmilk to administer orally to

the infant daily. The study intervention or placebo, stable in room temperature, will be given, in monthly supplies, from 4 to 24 weeks of age (total 20 weeks).

Strategies to improve adherence to interventions

To improve adherence, mothers will be issued diary cards and will also be requested to return empty sachets. Study staff will also contact mothers every 2 weeks through phone calls or in-person visits.

Relevant concomitant care permitted or prohibited during the trial

Infants can be prescribed concomitant medications based on the standard of care as necessary to provide adequate care and will be recorded. However, mothers will be asked to refrain from giving their enrolled infants cotrimoxazole prophylaxis or non-study probiotics, prebiotics, fibre or any nutritional supplements in the study period.

Outcomes

Primary outcomes (in infants HEU)

- ▶ Infectious morbidity from 4 to 24 weeks.
- ▶ Linear growth from 4 to 24 weeks.
- ▶ Infectious morbidity from 4 to 48 weeks.
- ▶ Linear growth from 4 to 48 weeks.

Infectious morbidity related to respiratory or gastrointestinal morbidity, data will be obtained from patient records and the Western Cape Provincial Health Data Centre (PHDC)¹⁸ sources for hospitalisations. Research assistants will conduct study participant interviews according to standardised case report forms through phone calls and study visits (and when available from patient records/PHDC sources) to capture infectious morbidity requiring outpatient primary care provider visit as well as infectious morbidity experienced at home (ie, those not requiring hospitalisations or primary care provider visits). For growth, infant anthropometry (weight and length) will be collected by study staff at each visit to assess length-for-age z-scores (LAZ; linear growth).

Secondary outcomes (in infants):

From 4–24 to 4–48 weeks:

- ▶ Gut microbiota composition, maturation and function.
- ▶ Faecal short-chain fatty acid levels.
- ▶ Plasma metabolome profile.
- ▶ Plasma levels of markers for inflammation and growth.
- ▶ Infant plasma HMO levels.
- ▶ Safety and tolerability of intervention.
- ▶ Severe infectious morbidity.
- ▶ Infectious morbidity and growth (for HEU vs HUU objectives).

The secondary endpoints are focused on hypothesised biological pathways through which the intervention works (detailed in the 'Data and sample collection' section). Other outcomes are related to the intervention safety and tolerability, severe infectious morbidity outcomes (ie, those requiring hospitalisation) as well as comparisons to infants HUU.

Participant timeline

Assessment of infant outcomes, along with other data and samples, will be collected at time points shown in Schedule of Evaluations (SoE) through 48 weeks of age (table 1) for infants HEU and HUU.

Sample size

This proof-of-concept futility study is designed to investigate whether the synbiotic has promise as an intervention to ameliorate common infant adverse outcomes and to investigate its effects on the hypothesised pathways of action including microbiota composition, maturity and function as well as inflammation. We propose a study of 184 mother-infant pairs (144 HEU and 40 HUU).

Based on data from our study population, we estimate that infants HEU on placebo will have a 50% probability of any infectious morbidity through 24 weeks, and 70% probability through 48 weeks. With a sample size of 63 in each group (ie, after accounting for 10% lost to follow-up and excluding around 4 infants HIV-exposed who are anticipated to acquire HIV), we have 83% and 88% power to reject the null hypothesis of superiority (ie, the probability of infectious morbidity is 20% points lower in the intervention group) at 0.10 significance level at 24 weeks and 48 weeks, respectively, and declare that the intervention is deemed futile (ie, not promising) if there exists no difference on infectious morbidity between the two groups in truth. This margin of superiority of 20% point difference is determined in line with previous effects of 2^oFL on infectious morbidity through 24 weeks of age.¹⁷ We set type I error at 0.10 as is conventional in proof-of-concept trials¹⁹

For differences in continuous measures of anthropometrics (LAZ and weight-for-length z-scores (WLZ)), we will be able to detect a difference in the mean LAZ (or mean WLZ) of intervention and placebo groups of ± 0.25 – 0.27 (assuming within-subject correlation 0.5–0.7) in LAZ/WLZ/weight-for-age z-scores (WAZ)/microbiota-for-age z-scores (MAZ) over 24 weeks or 48 weeks (a clinically significant difference,²⁰ with an 80% power to reject the null hypothesis at 0.10 significance level, assuming an SD of 1 LAZ (or WLZ/WAZ/MAZ). For differences at each cross-sectional time point, we will be able to detect a difference in the mean LAZ (or mean WLZ) of intervention and placebo groups of ± 0.31 .

Recruitment

Identification of potential study participants will occur at 3–7 days of age at primary healthcare clinics in and around the greater Worcester area, South Africa. Those who could be eligible for the study and are interested will be scheduled for further evaluation at 2 weeks at the Worcester Campus of SU when details of study participation will be explained, and study staff will obtain informed consent. After providing informed consent, women will be screened to confirm eligibility, and eligible participants will be enrolled in the study.

Assignment of interventions

At the entry visit in 4 weeks of age, enrolled participants will be randomised at a 1:1 ratio (intervention: placebo) using a permuted block design. Concealment of random assignment will be accomplished by creation of randomised treatment assignment list, based on permuted block size, by a statistician. The intervention and placebo will use identical sachets and labelling, and the placebo will have the same amount and similar taste/appearance as the intervention. Trial participants, on-site research team and care providers and outcome assessors will be blinded to the intervention.

Procedure for unblinding if needed

Unblinding will occur if there is clinical concern by the study team or the treating provider and they believe that unblinding the treatment would change the course of clinical care. Unblinding may also be necessary based on requests from ethics committees, Data Safety and Monitoring Board (DSMB) or the sponsor. For patients whose treatment assignment is intentionally unblinded, their treatment will be discontinued. Accidental or intentional unblinding will be recorded, and we will withdraw the participant from the study.

Data collection and management

Data and sample collection

Data collection will be done by the clinical trial staff at the SU site following the SoE in table 1. Maternal and infant clinical history (including HIV medications) and sociodemographic data will be collected using questionnaires. Detailed pregnancy history, delivery and birth data will be obtained through the mother's standardised maternity care document used at facilities across the province. Maternal CD4 count, HIV viral load and infant HIV PCR test results will be accessed by the study coordinator from the National Health Laboratory Service password-protected web-based interface. Infant and maternal anthropometrics (weight and length) will be collected by study staff at each visit. Data will also be collected on breastfeeding practices as well as infant neurodevelopment screening assessment. Outcome data related to infectious morbidity, growth, safety and tolerability will be collected as detailed above in the outcomes section. Building on data and lessons from our prior 1-month feasibility study of the placebo on 10 mother–infant pairs, we will also collect data on feasibility, acceptance, tolerability and behavioural adherence of both the intervention and placebo given for a longer period of time in a larger sample size. We plan to retain participants with anticipatory phone calls prior to visits, assistance with transport to the site, reimbursement for visits, and counselling and support.

Sample collection: Maternal blood, breastmilk, infant blood and stool samples will be collected as detailed in table 1. From maternal breastmilk, we will measure HMOs in mother's milk to assess the pre-existing HMO profile and its balance across groups. The infant samples will

be used to address secondary infant outcomes including (1) faecal gut microbiota composition and maturation (using 16S rRNA sequencing), (2) faecal short-chain fatty acid levels (including acetic acid, butyric acid and propionic acid), (3) plasma untargeted metabolome profile, (4) plasma inflammatory markers and growth hormones (using immunoassays) and (5) plasma HMO levels (using high-performance liquid chromatography methods).^{13–15} Examples of relevant immune and growth markers include those for intestinal fatty acid protein, lipopolysaccharide-binding protein, soluble CD14, (interleukin (IL)1b, IL6, tumour necrosis factor α , interferon γ -induced protein) and insulin-like growth factor 1.^{21–24}

Future use of samples for further testing may be conducted, and deidentified biological samples will be stored at the SU biorepository or the collaborating US labs.

Data management

All identified information will be kept in locked filing cabinets. All subjects enrolled in the study will be assigned a unique number that will be used in the electronic REDCap database. Data will be directly recorded in REDCap and only de-identified data will be shared with collaborators.

Confidentiality

Identifying information will be accessible only to the site principal investigator and co-investigator, and the study coordinator. All research activities will be conducted in as private a setting as possible.

Patient and public involvement

Patients or the public were not involved in the design, or conduct, or reporting, or dissemination plans of our research.

Statistical methods

Definition and statistical methods for primary and secondary outcomes

This is a proof-of-concept study to test the promise of the proposed intervention to reduce excess infectious morbidity and growth faltering. If either infectious morbidity or growth measures (LAZ, WAZ, WLZ) over either the 4–24 or 24–48 weeks time periods show promise, we would consider the intervention promising for further study. We will also consider shifts in the secondary outcomes of biomarkers as further supportive evidence.

Primary outcome

Analyses will follow intention-to-treat (ITT) principles comparing the cohorts as randomised for the four primary outcomes of infectious morbidity (1) 4–24 weeks; (2) 4–48 weeks) and linear growth (3) 4–24 weeks and (4) 4–48 weeks). We will compare arms with respect to the occurrence of binary (infectious morbidity) outcomes through 4–24 weeks and 4–48 weeks using a Wald test. We will compare arms with respect to continuous (LAZ)

outcomes over time using generalised linear mixed effects models (GLMM), accounting for repeat measures of the same participant over time. Separate analyses will be performed for the period from 4 to 24 weeks and from 4 to 48 weeks. Please note that the purpose of this futility trial design, in some manner, is to screen out unpromising treatments from further confirmatory testing. If the null hypothesis (stating the superiority of proposed intervention over the control) is not rejected (or equivalently, if the 95% CI overlaps with the magnitude of benefit specified in the sample size calculations), we will interpret the results as indicating ‘promise’, and a larger scale confirmatory trial will be recommended. However, if we reject the null hypothesis, in other words, the evidence suggests the proposed intervention is not promising, we will declare that the intervention is deemed ‘futile’, and no subsequent trial will be warranted.

Secondary outcomes

Measures:

- ▶ Infant growth
 - WLZ.
 - WAZ.
- ▶ Severe infectious morbidity (ie, requiring hospitalisation).
- ▶ Any severe illness (ie, requiring hospitalisation).
- ▶ Gut microbiome diversity, composition and maturity.
- ▶ HMO levels in infant plasma
- ▶ Faecal short chain fatty acids (SCFAs) levels.
- ▶ Plasma markers of inflammation and growth.
- ▶ Plasma metabolome.
- ▶ Above measures in infants HUU.

Analysis methods

For WLZ and WAZ outcomes, we will use similar analysis methods as for primary outcome analysis of LAZ to evaluate whether the intervention group has potential to be superior to the control in WLZ (or WAZ). For the rest of the secondary outcomes, we will test the traditional null hypothesis of no difference between the two group means or proportions using similar analytical approaches (eg, Wald test and GLMM).

For gut microbiome, we will compare α -diversity²⁵ (measured with Shannon and Simpson index) and microbiota maturity (measured as MAZ^{15 26} between intervention arms at each time point using t-test. We will also assess trends in alpha-diversity across the time points within each arm using linear regressions treating α -diversity measures as the outcome and time as the indicator. We will then compare time trends in alpha-diversity between two intervention arms by adding an interaction term time*intervention indicator in linear regressions. At each cross-sectional time point, we will also compare differences in individual taxon abundance using t-test adjusting for multiple comparisons.

At each time point, we will compare HMOs, metabolome, SCFAs and inflammation/growth markers between the arms using t-test and Mann-Whitney test adjusting for multiple

comparisons using Benjamini-Hochberg procedure to control for false discovery rate.²⁷

We will separately compare infectious morbidity and growth between (1) infants HEU on synbiotics versus infants HUU and (2) infants HEU on placebo versus infants HUU. We will use similar analytical methods as with primary objective 1. Since these are not ITT analyses, we will investigate, and adjust for potential confounders in GEE/GLMM models.

To investigate associations of biological parameters with infectious morbidity and growth outcomes in infants HEU and HUU, we will conduct assessments separately in each of the three cohorts (ie, HEU synbiotics, HEU placebo, HUU) and in the combined population (infants HEU and HUU).

Interim analyses

As this is a proof-of-concept study to test the promise of the proposed intervention focused on multiple outcomes, we will only stop the study early if the intervention is found to be futile on both primary outcomes: (1) infectious morbidity through 24 weeks and (2) LAZ at 24 weeks. The interim analysis will be conducted after two-thirds of the study participants have completed their 24-week visit. The monitoring plan will be discussed with and approved by the DSMB. We propose to use a modified Haybittle-Peto monitoring plan, with an interim stopping rule of z-score -3.0902 , corresponding to a nominal, one-tailed standard normal critical value cutting off total probability 0.001 in the lower tail. Thus, a strong signal of negative efficacy significant at the 0.001 one-tailed level (ie, the intervention is non-promising) would trigger DSMB consideration to recommend stopping the trial. The data from the interim analysis will be presented to the DSMB and the trial will be stopped early if it meets the above criteria.

Methods for additional analyses (eg, subgroup analyses)

Reports of digestive tolerance and behavioural patterns will be categorised as binary variables over time. Arms will be compared over time, measuring from baseline to week 24 using GLMM models. Safety analysis will also be conducted that will compare HIV infection of infants and adverse events (AEs).

Methods in analysis to handle protocol non-adherence and any statistical methods to handle missing data

The primary analysis will be conducted following ITT principle for which data collected from all the randomised patients will be analysed as the group they are randomised. Rubin's multiple imputation method will be employed to impute the missing endpoint for conducting the ITT analysis.²⁸ We will also conduct the per-protocol analysis to evaluate how protocol non-adherence impacts the magnitude of the intervention effects. Such analysis will allow us to better understand the findings from the primary ITT analysis.

Oversight and monitoring

Composition of the data monitoring committee, its role and reporting structure

A DSMB consisting of a paediatrician, statistician and two other clinician/public health scientists from Africa and USA has been convened to provide oversight. Membership of the DSMB is independent from the study and free of conflict of interest.

AE reporting and harms

Expected adverse intervention/placebo reactions:

No adverse reactions are expected from the placebo or intervention administration. Expected and unexpected AEs will be collected. The following are AEs that could be reasonably expected from this group of infants during the study:

- ▶ Infections and infestations including respiratory tract infections, diarrhoea (including intravenous rehydration), tuberculosis and otitis/ear infection.
- ▶ Fever, vomiting, spitting up and flatulence/gastrointestinal upset.
- ▶ Future healthcare provider visits.

Dissemination plans

Participants will be informed of study-related results on an aggregate level through knowledge translation activities in the final year. Additionally, study results will be presented in peer-reviewed journals.

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Acknowledgements We would like to thank the DSMB board for their helpful feedback: Drs. Lee Fairlie, Elizabeth Spooner, Jeannine Baumgartner and Sean Brummel. We would also like to thank IFF for the study product donation and packaging, and Sanopi for donation of feeding cups.

Contributors RS, ALS and LK led the conceptual design, and are leading the implementation, analysis and interpretation of the study. BL is leading the neurodevelopment assessments and is contributing to the study design and implementation of the study. MS and ES contributed to study design and lead the study implementation. RHG, LB and GA are leading laboratory assessments related to inflammation, HMOs and microbiome, respectively, and are contributing to the design and implementation of this study. SW and C-SL are leading the statistical analyses for this study and contributed to the study design and implementation. All authors contributed to manuscript writing and have approved the final manuscript and agreed to publication.

Funding This protocol and randomised trial is funded by the National Institute of Child Health and Human Development (award #: R01HD105492). The authors have ultimate authority over these activities.

Disclaimer The intervention and placebo used in this study were donated by IFF, the manufacturer of these products. IFF provided logistical support related to product use, including packaging and labelling. Other than that, IFF had no role in the study design, data collection, management, analysis, and interpretation of data; writing of the manuscript; and the decision to submit the manuscripts for the publication. The funding body (i.e. NIH) also has no role in the study design, data collection, management, analysis, and interpretation of data; writing of the manuscript; and the decision to submit the manuscripts for publication.

Competing interests None declared.

Patient and public involvement Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

Patient consent for publication Not applicable.

Provenance and peer review Not commissioned; peer reviewed for ethical and funding approval prior to submission.

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