Lactoferrin Infant Feeding Trial: LIFT
Statistical Analysis Plan

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1 Introduction

The aim of LIFT (Lactoferrin Infant Feeding Trial) is to determine the effectiveness of adding bovine lactoferrin (bLF) to the feeds of preterm babies of less than 1500 g birth weight. The key eligibility criteria are: <1500 g birth weight, ≤7 days old and expected to survive, and no severe congenital anomalies that are likely to cause death.

LIFT is a multicentre phase III trial that randomises eligible babies to the experimental group receiving bLF added to breast milk or formula milk once daily, or to the control group receiving no bLF added to breast milk or formula milk. These strategies (hereafter referred to as treatments) are intended to continue until 34 weeks corrected gestation or for 2 weeks, whichever is longer, or until discharge home, if earlier.

The randomisation is performed using a centralised system that allocates patients in a 1:1 ratio via minimisation stratified by site, gender, birth weight (<1000 vs ≥1000-1499g), and according to whether the baby is from a single or multiple birth.

LIFT is masked (i.e. treatment allocation is concealed from investigators, clinicians, parents, study personnel) however a designated person (e.g. bedside nurse, pharmacist, member of milk kitchen staff, or any appropriate staff member) prepares the milk feed containing either bLF (treatment group) or nothing (control group).

This analysis plan provides additional detail to the LIFT protocol (Version 2.0, 20JAN17, ACTRN12611000247976) on endpoint derivations and planned analyses. The health economic analyses described in the protocol are not detailed in this analysis plan.

2 Endpoints

The primary endpoint for the study is morbidity-free survival to hospital discharge. The key secondary short-term endpoints comprise the 5 components of the primary composite endpoint. The other secondary short-term endpoints are: time to reach full enteral feeds; number of blood transfusions; chronic lung disease; length of hospital stay; and, financial costs (out-of-scope for this analysis plan).

The following section provides detailed instruction on the derivation of endpoints. Any instances where the application of these instructions is unclear for a given baby will be resolved via blinded clinical review and documented.

2.1 Morbidity-Free Survival to Hospital Discharge (Primary Endpoint)

The primary endpoint is a composite of survival to hospital discharge (Section 2.2) free from:
(1) major morbidity at 36 weeks corrected gestational age defined as:

- brain injury (Section 2.3) or,
- necrotising enterocolitis of Grade II or higher (Section 2.4) or,
- late onset sepsis (Section 2.5); **AND**

(2) retinopathy treated according to local guidelines by discharge from hospital (Section 2.6).

Babies that survive to discharge but have missing data for any of the morbidities listed above will be assigned a missing value for the primary endpoint. Babies with no missing data for any of the morbidities AND who do not meet any of the morbidity criteria AND who survive to hospital discharge will be classified as having no event (i.e. event='No'). Babies that die prior to discharge and/or meet at least one of the morbidity criteria will be categorised as having had an event (i.e. event='Yes'). The estimated treatment effect will consequently reflect the impact of bLF on the likelihood of death and/or morbidity.

2.2 Survival to Hospital Discharge

Death is set to 'yes' if the following condition is met by data captured on the "Discharge (DISCHARGE)" eCRF:

- Was the baby alive at discharge? [itmSTATUS]='No'

2.3 Brain Injury at 36 Weeks Corrected Gestational Age

The endpoint 'Brain injury at 36 Weeks Corrected Gestational Age' will be derived using items from the "36 Weeks (OUTCOMES)" eCRF.

'Brain injury' will be set to 'Yes' if any of the following conditions are met:

- Worst grade of IVH seen (<14 day scan) [itmIVHGRADE] = ''Grade 3' or 'Grade 4'
- Echodense intraparenchymal lesions (>14 day scan) [itmIMECHO] = 'Yes'
- Periventricular leukomalacia (>14 day scan) [itmIMPERI] = 'Yes'
- Porencephalic cysts (>14 day scan) [itmIMPOREN] = 'Yes'
- Ventriculomegaly (97 percentile plus 4mm) (>14 day scan) [itmIMVENTRI] = 'Yes'
- Hydrocephalus (>14 day scan) [itmIMHYDRO] = 'Yes'
- Encephaloclastic porencephaly (>14 day scan) [itmIMENCEPH] = 'Yes'

'Brain injury' will be set to 'missing' if:

- the '<14 day scan' is performed >1 day late AND there is no grade 3-4 IVH identified; OR,
- the '>14 day scan' is performed >1 day early AND none of the criteria applicable to this scan listed above are met; OR,
- the '>14 day scan' is performed > 7 days after Week 36 AND deemed invalid on blinded clinical review.

'Brain injury' will be set to 'No' if none of the conditions above apply.

Other types of brain injury recorded as free text in the database will undergo a blinded clinical review against the criteria above.

2.4 Necrotising Enterocolitis of Grade II or Higher at 36 Weeks Corrected Gestational Age

The endpoint 'Proven episode of stage II NEC Higher at 36 Weeks Corrected Gestational Age' will be derived using items from the "36 Weeks (OUTCOMES)" eCRF. The table below indicates how the eCRF items (shown in columns A and B) will be used to determine NEC status (column C).

(A) Did the baby have a proven episode of necrotising enterocolitis [itmNEC]		(B) Please specify stage of disease [itmNECSTAGE]	(C) Proven episode of stage II or III NEC
No		-	No
Yes	AND	Stage I	No
Yes	AND	Stage II or III	Yes

2.5 Late Onset Sepsis at 36 Weeks Corrected Gestational Age

The endpoint 'Late onset sepsis (LOS) at 36 Weeks Corrected Gestational Age ' will be derived using items from the "36 Weeks (OUTCOMES)" eCRF. The table below indicates how the eCRF items (shown in columns A and B) will be used to LOS status (column C).

(A) Did the baby have one or more episodes of infection proven [itmSEPSISYN]		(B) Date sample taken [itmSAMPDATE]	(C) LOS
No		-	No
Yes	AND	No more than 7 days beyond the week 36 target	Yes
Yes	AND	More than 7 days beyond the week 36 target	No

2.6 Treatment for Retinopathy (to Discharge)

The endpoint 'Treatment for Retinopathy to Discharge' will be directly derived from the item 'Was the baby treated for ROP?' [itmROPTREATYN]) on the "Discharge (DISCHARGE)" eCRF.

2.7 Time to Full Enteral Feeds

Time to full enteral feeds is measured from the date of randomisation to the date recorded in the eCRF corresponding to the 3rd day on which enteral intake of ≥ 120 ml/kg/day was first achieved for 3 consecutive days.

2.8 Number of Blood Transfusions to 36 Weeks Corrected Gestational Age

The number of transfusions to 36 weeks corrected gestational age is assessed by the question below appearing in the "36 Weeks (OUTCOMES)" eCRF:

- How many transfusions of whole blood or packed cells? [itmNUMTRAN]

The number of transfusions will be set to 0 if the following item is answered 'No':

- Did the baby have a transfusion before 36 weeks? [itmTRANYN]

2.9 Chronic Lung Disease at 36 Weeks Corrected Gestational Age

The endpoint 'Chronic lung disease at 36 Weeks Corrected Gestational Age ' will be derived using items from the "36 Weeks (OUTCOMES)" eCRF. The table below indicates how the eCRF items (shown in columns A to C) will be used to derive chronic lung disease status (column D).

(A) Did the baby receive respiratory support at any time? [itmVENT]	(B) Is the baby still on supplemental oxygen at 36 weeks? [itmVENT36WKS]		(C) "Date last on supplemental oxygen or on assisted ventilation" [itmVENTDATE]	(D) Chronic lung disease
No	-		-	No
-	No		-	No
-	Yes	AND	No more than 7 days before week 36 target	Yes
-	Yes	AND	More than 7 days before week 36 target	No

2.10 Length of Hospital Stay

Hospital length of stay is measured from the date of birth to the date the baby is discharged from hospital.

2.11 Survival and Development Outcomes at 24 to 36 Months Corrected Gestational Age

Major disability is defined as meeting any of the criteria below based on an evaluation performed during the assessment window using the instruments below;

- a modified Short Health Status Questionnaire (SHSQ) completed by a medically qualified practitioner documenting:

a) major developmental delay, including language or speech problems, or
b) cerebral palsy with inability to walk unassisted, or
c) severe visual loss (cannot fixate/ legally blind, or corrected acuity <6/60 in both eyes) , or
d) deafness, requiring a hearing aid or cochlear implants.

- A cognitive composite score from the Bayley-III Scales of Infant and Toddler Development (BSID) indicative of least moderate delay (i.e. a score at least 2 SDs below the age-adjusted norm on the index)
- A problem solving score from the Ages and Stages Questionnaire (ASQ) indicative of least moderate delay (i.e. a score at least 2 SDs below the age-adjusted norm on the index. Note that a LIFT substudy will, blinded to treatment, compare available BSID data with corresponding ASQ data to ensure the chosen ASQ score cut-point corresponds adequately well to that for the BSID).

Survival status is collected at discharge and annually thereafter. An infant will be classified as being alive and free from major disability if none of the criteria above are met and adequately complete information is obtained during the assessment window from at least one of the instruments. Any unclear cases (e.g. where only partial information is available, a death occurs after the date of the developmental assessment and before month 36, etc.) will be resolved on blinded clinical review of available information.

2.12 Safety

In addition to the morbidity endpoints defined above, safety will be assessed according to the incidence of suspected adverse reactions that are classified as serious and unexpected (Suspected Unexpected Serious Adverse Reactions – SUSARs).

3 Analysis Sets

- The ITT population will comprise all randomised participants.
- The Per Protocol population will comprise all randomised participants that receive least one administration of their assigned treatment and who are not deemed ineligible on blinded clinical review.
- The safety population will comprise all randomised participants who received at least one administration of assigned treatment. Participants will be analysed according to the treatment they actually received for the purposes of the safety analysis.

Any instances where membership of an analysis set is unclear will be resolved on blinded clinical review. Any exclusions from analysis sets will be documented.

4 Interim and Final Analyses

The LIFT protocol makes provision for interim analysis of the primary composite endpoint, and of survival to discharge, using the Haybittle–Peto approach (see Peto et al[1], Statistical Note No. 4, p. 611). The procedure involves evaluating the test statistic calculated under null hypothesis against a boundary of 3 standard deviations (equivalent to $\chi_1^2 = 9$, with p-value of 0.0027). This has a negligible effect on the alpha level applicable at final analysis (i.e. the analysis performed when the boundary is not crossed at any interim analysis and the study continues as originally planned).

5 Type I Error (Alpha)

A two-sided alpha (significance level) of 5% will be applied to the analysis of the primary composite endpoint. There will be no adjustment to alpha for interim analyses evaluated using the Haybittle–Peto boundary.

The key secondary endpoints for LIFT comprise the individual components of the primary composite endpoint (i.e. survival, brain injury, NEC, LOS, and RoP). P-values adjusted for the five comparisons performed for this set of endpoints will be derived using Benjamini-Hochberg procedure[2] with a family-wise error rate of 5%. Results of other endpoint, subgroup, and sensitivity analyses will be interpreted in proper context and with due consideration of type I error. The clinical interpretation of the statistical evidence from LIFT will take into account the recommendations of Pocock et al[3,4].

6 Analysis of Study Endpoints and Patient Characteristics

Safety analyses will be performed using the safety population. Non-safety analyses will be performed using the ITT population. Sensitivity analyses may be performed using the Per Protocol population.

6.1 Subject Disposition

The number of patients in each analysis set will be presented along with reasons for any exclusions.

6.2 Baseline Demographic and Clinical Characteristics

Descriptive statistics will be prepared to summarise the baseline characteristics of the study participants by treatment allocation. Variables to be summarised include: gender, multiple birth, birth weight, gestational age at birth, inborn/outborn, mode of delivery, and APGAR scores at 1 and 5 minutes.

6.3 Duration of Study Treatment

The number of days study treatment is delivered will be summarised by treatment group using descriptive statistics. Summaries by treatment group will also be prepared for the proportion receiving treatment for at least 1 week, and proportion receiving treatment for at least 2 weeks.

6.4 Morbidity-Free Survival to Hospital Discharge (Primary Endpoint)

The primary analysis will be a comparison between treatment groups on the proportion of babies experiencing the primary composite endpoint that is tested using a Wald test (with a χ_1^2 distribution) from a log-binomial model fitted using generalised estimating equations to accommodate possible correlation of data between siblings from multiple births. If the log-binomial model does not converge, a logistic model will be used.

A multiple imputation approach will be used to account for missing primary endpoint data in a sensitivity analysis. The effect of assuming all missing values are uniformly indicative of an 'event' (pessimistic scenario) or of 'no event' (optimistic scenario) may also be explored.

Estimates of the treatment effect on the primary endpoint that are adjusted for stratification factors and adherence to assigned treatment will be produced using the methods detailed in Section 8.

6.5 Analysis of Categorical Secondary Endpoints

Secondary categorical endpoints include: survival to hospital discharge (Section 2.2), brain injury (Section 2.3), NEC (Section 2.4), LOS (Section 2.5), RoP (Section 2.6), chronic lung disease (Section 2.9), and survival and development status at 24-36 months corrected gestational age (Section 2.11).

Secondary categorical endpoints will be analysed using the same modelling approach applied to the primary endpoint (i.e. a log-binomial model fitted using generalised estimating equations to accommodate possible correlation of data between siblings from multiple births. If the log-binomial model does not converge, a logistic model will be used).

Sensitivity analyses will be undertaken on the categorical morbidity endpoints (i.e. brain injury, NEC, LOS, RoP, and chronic lung disease) in which any missing values due to death are treated as an event. An analysis of survival to 36 weeks may also be undertaken as a sensitivity analysis. Estimates of the treatment effect on survival to hospital discharge that are adjusted for adherence will be produced using the methods detailed in Section 8.2 in a sensitivity analysis.

6.6 Analysis of Quantitative Secondary Endpoints

Number of blood transfusions (Section 2.8) will be treated as count data and analysed using a negative binomial regression model fitted using generalised estimating equations to accommodate possible correlation of data between siblings from multiple births.

Time to full enteral feeds (Section 2.7) and length of hospital stay (Section 2.10) will be analysed using a linear regression model fitted using generalised estimating equations to accommodate possible correlation of data between siblings from multiple births. An appropriate data transformation will be applied if continuous data are highly skewed. Based on a blinded review of interim data, a log transformation of time to full enteral feeds is anticipated to be appropriate, whereas the distribution of length of hospital stay is expected to be reasonably symmetric.

The following supplementary binary endpoints will be derived and analysed to accommodate instances where data on enteral feeding, transfusions, or length of hospital stay are missing/truncated due to death:

- Baby reached full enteral feed milestone within 7 days of randomisation (No versus Yes)
- Baby survived with no transfusions to 36 weeks GA (No versus Yes)
- Baby was discharged alive within 2 months of birth (No versus Yes)

These supplementary binary endpoints will be analysed as part of a sensitivity analysis using the modelling approach applied to the primary endpoint (i.e. a log-binomial model fitted using generalised estimating equations to accommodate possible correlation of data between siblings from multiple births. If the log-binomial model does not converge, a logistic model will be used).

6.7 Safety Data Analysis

A descriptive analysis of SUSAR data will be conducted by treatment allocation. The event rate is expected to be low, and thus an exact test would be an appropriate choice to perform any formal comparisons between the two groups in the proportion experiencing a SUSAR.

7 Subgroup Analyses

Consistency of the treatment effect on the primary endpoint across subgroups will be tested by including a treatment-by-subgroup interaction term (along with the relevant main effect terms) as covariates in the analysis model (as specified in Section 6.4).

The subgroups of interest are: (i) birthweight <1000 g versus 1000-1499 g; (ii) randomised ≤72 hr versus >72 hr from birth; (iii) those who received versus did not receive probiotics by 36 weeks corrected gestation (note: this is a post-baseline covariate); (iv) ≤ 28 weeks versus >28 weeks gestation at birth. Because 'probiotic use by week 36' is a post-baseline covariate, an unbiased evaluation of its role as an effect-modifier will be attempted by categorising participating sites (NICUs) into tertile subgroups according to their propensity to use probiotics, and evaluating consistency of the treatment effect across these sub-groups.

8 Adjusted Analyses

8.1 Stratification Factors

Sensitivity of results obtained from the primary analysis of the primary endpoint to adjustment for stratification factors will be explored by included them as covariates in the model (See Section 6.4).

Randomisation is stratified by gender, birth weight (<1000 g vs ≥1000-1499 g), according to whether the baby is from a single or multiple birth, and site (NICU). Given the modest number of patients at some sites, and the degree of variation in the number of babies recruited across sites (i.e. minimum is less than 10 babies and the maximum is more than 200 babies), sites will be categorised into larger groups that have a comparable pooled primary endpoint event rate. This will be done by estimating, in a blinded fashion, the event risk at each site pooled across treatments, and deriving 'risk classification' according to tertiles. Risk classification will be used as the covariate in the adjusted analyses in preference to 'individual site'.

8.2 Treatment Compliance

An overall treatment adherence adjusted estimate of the effect of bLF on (i) the primary composite endpoint and (ii) survival to hospital discharge will be calculated by dividing the estimated relative risk reduction (RRR) by the adherence rate as per the equation below.

$$\text{RRR}_{\text{adjusted}} = \frac{\text{RRR}}{100\% - (\text{bLF nonadherence \%}) - (\text{PBO nonadherence \%})}$$

The non-adherence percentage in each arm is defined as the proportion receiving less than two weeks of allocated treatment for reasons other than death.

A second approach for deriving treatment adherence adjusted estimates will involve clustering sites achieving comparable adherence rates into tertiles, calculating the treatment effect for each tertile, deriving a weighted combination of these individual estimates where the weights reflect the degree of adherence achieved for the tertile, and combining the treatment effect within each tertile (estimated using the analysis approach specified in Section 6.4) with its corresponding adherence weight to derive a weighted estimate.

The adherence weight (w_i) for the i^{th} tertile comprising k sites with n_j babies per site is:

$$w_i = \frac{\sum_{j=1}^{k}\{n_j \times (\%\ Adherence_j)\}}{\sum_{j=1}^{k} n_j}$$

The weighted estimate, on a $\log_e$ scale, (G) is:

$$G = \frac{\sum_{i=1}^{3} w_i \times \log_e(RR_i)}{\sum_{i=1}^{3} w_i}$$

The variance (V) of G will be calculated as:

$$V = \frac{\sum_{i=1}^{3} {w_i}^2 \times \text{Var}(\ln(RR_i))}{(\sum_{i=1}^{3} w_i)^2}.$$

The point estimate and 95% CI will be reported as e^G and $e^{G \pm 1.96 \times \sqrt{V}}$ respectively.

9 Synthesis of Trial Evidence

The evidence from LIFT will be placed in broader context in the primary reporting of results by combining, where feasible, with evidence from other sources as described below.

9.1 Cochrane review

Consistent with the recommendation that reports of clinical trials should begin and end with an up-to-date systematic review of all available randomized evidence,[5,6] data from this trial will be used to update the most recent peer-reviewed Cochrane systematic review of trials of lactoferrin supplementation in preterm infants.[7] This will involve deriving an updated meta-analysis estimate of the effect of lactoferrin on late onset sepsis, necrotizing enterocolitis and all-cause mortality, to be summarised in a figure in the primary reporting of results. A meta-analysis of the primary composite endpoint used in LIFT is not currently possible because that composite endpoint has not been reported by the other trials.

9.2 LIFT_Canada

The protocol for the Australia and New Zealand (ANZ) LIFT trial was used to develop a similar Canadian trial (LIFT_Canada: ClinicalTrials.gov identifier NCT03367013) with a sample size target of N=500. Individual patient data from LIFT_Canada is planned to be combined with that from LIFT (the studies use identical electronic Case Report Forms) in order to perform pooled analyses. The pooled analyses will follow the methods described in this document with the following modifications: (1) the method for aggregating sites (NICUs) described in Section 8.1 will be implemented separately for ANZ and Canada; and, (2) evidence of heterogeneity of treatment effect across region (i.e. ANZ versus Canada) will be evaluated on the primary endpoint.

The timing of this pooled analysis will depend on when LIFT-Canada data becomes available. This is expected in late 2020, about 2 years after the release of LIFT Australia and New Zealand results, based on current projections. The pooled results will be included in a further update of the Cochrane review of enteral lactoferrin supplementation in preterm infants.

10 References:

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