The University of Sydney

NHMRC Clinical Trials Centre

Lactoferrin Infant Feeding Trial

Does lactoferrin improve survival free from morbidity in very low birth weight infants?

Lactoferrin Infant Feeding Trial: a randomised controlled trial

Protocol Version 2.0

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Table of Contents

SYNOPSIS

Primary clinical hypothesis:

Adding bovine lactoferrin (bLF) to feeds improves health outcomes in preterm infants.

Study objectives:

To test the hypothesis that adding bovine lactoferrin (bLF) to feeds in preterm babies of less than 1500 g birth weight will: (i) improve survival to hospital discharge free from brain injury, late onset sepsis, necrotising enterocolitis, and retinopathy of prematurity (primary composite endpoint); and, (ii) have a beneficial effect on each of the components of the primary composite endpoint as well as time to reach full enteral feeds, number of blood transfusions, chronic lung disease, and length of hospital stay.

To conduct a Cost Effectiveness Analysis of bLF in improving survival free from major hospital morbidity (in Australia only).

To evaluate the effect of bLF on survival and developmental outcomes at 24 to 36 months corrected gestational age.

Study design:

Multicentre 2-arm, randomised controlled trial.

Study interventions:

The two arms of the trial comprise:
(a) Treatment group: bLF added to breast milk or formula milk once daily
(b) Control group: no bLF added to breast milk or formula milk
Study intervention is administered until 34 weeks corrected gestation or for 2 weeks, whichever is longer, or until discharge home, if earlier.

Study population: Babies with birth weight less than 1500 g

Primary outcome:

Survival to hospital discharge free from (i) 3 morbidities diagnosed or treated in hospital by 36 weeks corrected gestational age: brain injury *or* late onset sepsis *or* necrotising enterocolitis (NEC); and, free from (ii) retinopathy treated according to local guidelines up to discharge from hospital.

Secondary outcomes:

Survival to hospital discharge, brain injury (to 36 weeks corrected gestational age), late onset sepsis (to 36 weeks corrected gestational age), NEC (to 36 weeks corrected gestational age, retinopathy treated according to local guidelines up to discharge, time to full enteral feeds, number of blood transfusions to 36 weeks, chronic lung disease (to 36 weeks), length of hospital stay, and survival and development outcomes at 24 to 36 months corrected gestational age.

Power and Sample Size:

A trial of 1,500 participants yields 85% power at the two-sided 5% significance level to detect a difference in the proportion meeting the primary outcome assuming the true probability is 74% in the control group and 80.5% in the bLF group.
Pre-defined subgroups:
Consistency of the treatment effect on the primary endpoint will be evaluated across the following subgroups:
(i) birth-weight <1000 g and 1000-1499 g;
(ii) randomised ≤72 hr and >72 hr from birth;
(iii) those who received or did not receive probiotics
(iv) ≤ 28 weeks and >28 weeks gestation

Concurrent Trials:

LIFT is compatible with concurrent trials in similar infants. Indeed, simultaneous participation in concurrent trials is encouraged because it increases (i) overall efficiency, as fewer total patients are needed to answer more than one question; (ii) the generalisability of the results of each trial and improves our understanding about the joint use of each treatment, (iii) reimbursement and funding for local research infrastructure and employment of research nurses at participating sites.

1 BACKGROUND INFORMATION

Prevention of death and disability in preterm infants is an urgent public health problem

Every year about 3,000 preterm infants with very low birth weight (VLBW: <1,500 g) are treated in Australia and New Zealand.[1] These babies are at elevated risk of death and major morbidities (Table 1) including severe brain or lung injury, retinopathy, late-onset sepsis or necrotizing enterocolitis (NEC), each of which is associated with substantial risk of childhood disability.[2-4]

Table 1: Outcome in 7, 957 VLBW infants admitted to ANZNN NICUs in 2005-2007‡

	Total	Cases	%
Death in hospital after 48 hours	7,957	607	7.6
Brain injury ‡‡	7,602	526	6.9
Chronic Lung Disease at 36 weeks‡‡	7,786	1, 660	21.3
Retinopathy Grade III or higher ‡‡	6,314	377	6.0
Late onset sepsis**	7,511	1, 293	17.2
NEC Grade II-IV‡‡	7,888	347	4.4

*‡‡ Defined as in ANZNN Data Dictionary (see text); ** occurring >48 h after birth;*

Lactoferrin is an antimicrobial, antioxidant, anti-inflammatory iron-carrying, bifidogenic glycoprotein found in all vertebrates and in mammalian milk, leukocytes and exocrine secretions.[5, 6] Most VLBW infants receive insufficient human lactoferrin (hLF) from breast milk in the first month, resulting in suboptimal protection.[7-10] In a three-arm RCT in 472 VLBW infants led by Dr Paolo Manzoni, [8-10] who is one of the Chief Investigators in LIFT, a fixed daily dose of 100 mg bovine lactoferrin (bLF) added to feeds for 4 – 6 weeks reduced the risk ratios (Relative Risks or RRs) of late onset sepsis, sepsis-related mortality, NEC and severe ROP by about 65%, with a trend to a two-thirds reduction in RR of hospital mortality. Importantly, bLF reduced late-onset sepsis in infants fed breastmilk, suggesting that its protective effect was independent of breastmilk. (Table 2) [8] The fixed dose of 100 mg equates to a range of 50 mg/ kg per day (in a baby of 2000 g) to 200 mg/ kg/ day (in a baby of 500 g). The RR of a combination of outcomes‡‡ similar to the composite outcome to be studied in LIFT was reduced by 50%, from 28% to 14.4% (Table 2), equivalent to an improvement in survival free from morbidity from 62% to 85.6%.

Table 2: Data from the only previous RCT of bovine lactoferrin (bLF) in VLBW infants ‡ [8-10]

Outcome	Placebo (%)	bLF (%)	Risk Ratio (95% CI)	P
Death in hospital after day 3	12/168 (7.1)	4/153 (2.6)	0.37 (0.12 - 1.11)	0. 07
Hospital death from late-onset sepsis	8/168 (4.8)	0/153 (0)	NA	0.008
Late-onset sepsis (bacterial + fungal)	29/168 (17.3)	9/153 (5.9)	0.34 (0.17 - 0.70)	0.002
Late-onset sepsis (bacterial only)	23/168 (13.7)	9/153 (5.9)	0.43 (0.21 - 0.88)	0.02
Late-onset sepsis in infants fed breastmilk (not exposed to formula)	7/37 (18.9)	1/42 (4.2)	0.13 (0.02 - 0.74)	0.02
Necrotising enterocolitis (≥ stage 2) [9]	14/259 (5.4)	5/251 (2.0)	0.37 (0.13 - 0.99)	0.04
Retinopathy of prematurity (treated)	19/168 (11.3)	6/153 (3.9)	0.35 (0.12 - 0.82)	0.02
Death >day 3 or severe morbidity‡‡	47/168 (28.0)	22/153 (14.4)	0.51 (0.32 - 0.80)	0.003

‡ only bLF and placebo are shown;

‡‡ Death after day 3 or brain injury or chronic lung disease or treated ROP or late-onset sepsis or NEC

Why is this new trial needed?

Early trials can overestimate treatment effects. A Cochrane Review [11] called for these results[8]

"…to be confirmed in well designed, adequately powered, multi-centre trials with higher dosage and longer duration of treatment, addressing safety, the impact of human milk feeds and later disability," … as *"current evidence is insufficient to support a change in practice"*.

In March 2012, 96% (22/ 23) of Australian and New Zealand Neonatal Network (ANZNN) neonatal intensive care units (NICUs) did not use bLF. Clinicians are thus cautious about introducing routine bLF after one study in 472 VLBW infants.[8] However, all 18 NICUs which expressed formal interest in participation indicated that they will change practice if LIFT shows bLF to be associated with a 25% reduction in death or major morbidity . LIFT is adequately powered to detect an effect this size, which is clinically important, plausible, and more conservative than the earlier trial result. LIFT also addresses key issues raised by the Cochrane Review.

If LIFT confirms an effect of this magnitude, bLF will have major impact in this setting, translating into about 200-300 additional intact survivors without major morbidity in Australia and New Zealand every year and many more in North America, Europe and worldwide. Because >90% very preterm survivors at hospital discharge reach adulthood,[12, 13] this represents well in excess of 13,000-19,000 life-years gained in Australia and New Zealand each year, which would be one of the largest gains in intact survival in any specialty since neonatal surfactant and antenatal steroids.[14, 15]

Potential mechanisms of action and benefits of lactoferrin (human and bovine)

Lactoferrin is a cationic glycoprotein found in milk, tears, saliva, sweat, cerebrospinal fluid and neutrophils. It is present on mucosal surfaces and is part of the innate immune response.[5] Plasma levels of lactoferrin are low in preterm infants, but rise in response to infection.[16] Lactoferrin is the major whey protein in human colostrum (~7 mg/mL) and mature breast milk (~2 mg/mL).[17]

- ***Lactoferrin is a potent antimicrobial agent***

Lactoferrin is a potent inhibitor and microbicide for bacteria, fungi, viruses and protozoa. Several modes of action are described, e.g. sequestering iron (an essential substrate for pathogens); disrupting bacterial and fungal cell membranes; inhibiting microbial adhesion to host cells; preventing biofilms; blocking viral entry and transmission between host cells; inhibiting protozoal growth[18-22] and promoting the growth of bifidobacteria in the gut. [23] Human lactoferrin (hLF) has a molecular weight of 80 kilodaltons and shares 77% amino acid homology with its isoform, bLF. [24] The activity of bLF against Gram negative bacteria is partly mediated by its positively charged N-terminal peptide which binds to negatively charged lipopolysaccharide (LPS) in the bacterial membrane, inactivating surface anchored type III secretory system virulence proteins.[18]

"Multiple simultaneous mutations might be required for emergence of lactoferrin resistance ... This complexity may explain lactoferrin's continuing biologic activity despite eons of interactions with bacteria. Alternatively, bacterial resistance might be achieved by changing the structure of LPS; however, this may be equally problematic from the bacteria's point of view given the multitude of surface anchored proteins that must interact with LPS."[18]

Lactoferrin also binds to lipoteichoic acid in Gram positive bacterial membranes. [25] Lactoferrin undergoes partial acid proteolysis in the stomach to yield lactoferricins, which are peptides with enhanced antimicrobial activity.[26] This may partly explain why H_2 antagonists, which suppress gastric acid and proteolysis in the stomach, are associated with increased risk of sepsis.[27]

- ***Bovine lactoferrin stimulates gut maturation and enhances the growth of probiotic organisms***

At high concentration, as in colostrum, bLF causes proliferation of enterocytes and closure of enteric gap junctions, decreasing 'leaky gut' syndrome by reducing permeability to bacteria and loss of fluid. [28] At lower concentrations, bLF causes differentiation of enterocytes and the expression of enzymes such as lactase, enhancing digestion.[28] Also, bLF enhances the growth of probiotic organisms like Bifidobacteria in the gut, [23] which improves tolerance to feeds and reduces NEC. [29]

- ***Anti-inflammatory and anti-oxidant effects on sepsis, ROP, NEC and lung and brain injury***

The anti-inflammatory and anti-oxidant properties of lactoferrin [30-35] could explain the striking reductions in severe ROP and NEC seen in Table 2. [8-10] The anti-inflammatory activity of hLF is illustrated by its ability to prevent gut injury in experimental colitis.[32] Also, hLF is an anti-oxidant that reduces free radical formation and lipid peroxidation caused by adding iron to breastmilk or formula. [33]

- ***Many VLBW infants cannot ingest optimal quantities of human lactoferrin***

Owing to feed intolerance or illness, many VLBW infants never reach recommended intake, resulting in what has been called "inevitable postnatal malnutrition".[7, 17, 36-38]

- ***Early supplementation with bovine lactoferrin compensates for low intake of hLF***

Because of its high molecular weight, 100mg of bLF has an acceptable osmolality of <390 mosm/L when dissolved in 1ml of milk, (Manzoni, unpublished data). When bLF was given in small volumes in our earlier study, [8] most VLBW infants achieved daily intakes of 100 – 170 mg/ kg within 24 hours after starting trophic (minimal) feeds, significantly boosting their total intake of lactoferrin. The clinical benefit observed previously [8] may thus simply reflect a higher dose-response to total lactoferrin intake.

- ***Bovine lactoferrin may also increase the total volume of breast milk received***

By reducing episodes of feed intolerance, suspected or proven sepsis and NEC, bLF prophylaxis may also increase the total volume of breast milk received, amplifying its protective effects.

- ***Bovine lactoferrin may reduce the number of blood transfusions given***

bLF is a natural iron supplement which, when added to milk formula, was associated with higher haematocrit levels at 9 months in a RCT in term infants. [39] Accordingly, bLF may result in fewer episodes of late anaemia and fewer blood transfusions.

- ***Potential reductions in hospital stay***

Lastly, late onset sepsis occurs in ~17% of VLBW infants (Table 3), adversely impacting costs and service delivery. VLBW infants with sepsis spend nearly 3 weeks longer in hospital.[40] If bLF significantly reduces sepsis, it may shorten hospital stay.

Need for new strategies to reduce late onset neonatal sepsis

All participating NICUs will be required to maintain active programmes to promote hand hygiene and high standards of infection control and aseptic technique. However, while such measures reduce hospital infections, rates of infection tend to reach a plateau, despite intensive implementation of these practices.[41] This re-emphasizes the need for novel strategies to prevent sepsis, such as bLF. [8-10, 18]

Safety issues

bLF is integral to the human diet because it is present in milk at a concentration of ~0.4 mg/mL.[42] It is registered as GRAS (Generally Recognised As Safe) by the US FDA, with no known toxicity.[43] In rodents the no-observed-adverse-effect level (NOAEL) of bLF was at least 2,000 mg/kg/day.[44] Although bLF has an excellent safety record and no allergic or adverse reactions occurred in our previous trial, [8-10] there is a theoretical risk of cow's milk allergy in later childhood.

Questions that were considered in the design of the trial

- ***Why have infants of <1,500 g birth weight been selected as the target group?***

Infants of <1,500 g have a higher incidence of late onset sepsis and NEC than infants of <32 weeks gestation. [1] This target group, defined by birth weight, therefore provides greater power. Subgroup analysis is planned for infants ≤ 28 weeks and >28 weeks gestation.

- ***Why has all-cause mortality not been selected as the primary outcome?***

In a therapeutic Phase 2 RCT in 194 adults with severe sepsis, recombinant human lactoferrin reduced 28 day all-cause mortality from 26.9% to 14.4% (p = 0.052) [45], equivalent to an increase in total survival from 73.1% to 85.6%. Total survival was considered as a primary outcome in the current prophylactic RCT in VLBW infants but, because the total survival rate is much higher, to show an increase in survival, from 92.5% to 95%, with 80% power requires about 3,000 infants, which is financially prohibitive. However, LIFT will contribute, with a UK RCT of bLF led by W. McGuire and with other RCTs, to a pooled overview in >4,000 VLBW infants, with greater power to address total survival.

- ***Rationale for the primary composite outcome: net clinical benefit, safety and effectiveness***

Composite neonatal outcomes are frequently used in multicentre trials, including several funded or co-funded by NHMRC, [46-53] because parents, surviving children and clinicians place a high value on intact survival free from major morbidity.[54] The primary outcome in LIFT is thus a composite of survival from enrolment to discharge free from (i) any of three morbidities diagnosed or treated in hospital by 36 weeks gestational age: brain injury or late onset sepsis or necrotising enterocolitis (NEC); and free from (ii) retinopathy (ROP) treated before hospital discharge.

Recommended approaches for constructing this primary composite outcome were followed and will be applied in analysis and interpretation of results.[55, 56] All morbidities have been pre-specified [2-4] and each is independently associated with later childhood disability. [2-4] The primary composite outcome is thus a rational proxy for net clinical benefit. It is also amenable to unbiased assessment. [55, 56]

Lastly, each of these component morbidities has been linked with free radical disease. [30-35] This supports the hypothesis that each will be affected in a similar direction, because of the anti-oxidant, anti-inflammatory and antimicrobial properties of bLF.

One potential concern is that not all the composite elements are equally important. Each will thus also be analysed individually as a secondary outcome, following published recommendations.[55, 56]

- ***What effect will increasing use of probiotics have on the trial?***

Because probiotics reduce the relative risks of NEC and all-cause mortality by 60%,[29] many clinicians are considering routine prophylaxis with probiotics.[57] However, there is little or no evidence that probiotics improve sepsis or retinopathy,[29] in contrast to bLF.[8-10] Also, bLF has multiple mechanisms of action, so its impact on NEC is probably independent of probiotics.

In a subgroup analysis, the effect of bLF vs control will be assessed in infants who were and were not treated with probiotics by 36 weeks corrected gestation. To have a conservative estimate of the

trial's sample size and power, baseline rates of NEC and all-cause mortality in the primary composite outcome have been adjusted down, to account for the potential effect of probiotics.

In view of the evidence for probiotics and to reduce the risk of potential co-intervention bias, participating centres will be asked to provide the trial co-ordinating centre with a copy of their current probiotics protocol, if they have one.

Confirmation that bLF reduces severe ROP (Table 2) [8-10] will be of added importance because recent evidence from the US and our group,[58, 59] is shifting global practice to higher oxygen saturation targets, which may increase the incidence of severe ROP. [58]

- ***Why not evaluate human lactoferrin?***

Human lactoferrin (hLF), produced by recombinant gene technology (Talactoferrin), is patented and under evaluation in all age groups in placebo-controlled RCTs by Agennix, a multi-national biopharmaceutical company. These include trials in four US NICUs in 120 VLBW infants, and in five Peruvian NICUs in 634 infants of birthweight <2000 g. A commercial hLF product will be more expensive than bLF, which is not patented. The retail price of bLF is <$1 per 100 mg.

There are no published RCTs in preterm infants supporting the use of hLF. Our earlier trial [8] provides the best evidence that lactoferrin is of clinical benefit and shows that bLF has an excellent safety profile, making recombinant hLF potentially unnecessary. There is evidence that bLF may be more effective than hLF, for example in stimulating enterocyte growth and proliferation.[28] However, even if the benefits of bLF effect only reflect a dose – response to total lactoferrin intake, LIFT will explore this, as called for by the Cochrane Review, [11] by using a larger dose in this study (i.e. up to 250 mg/ kg/ day) than in our previous study, which used an average of ~100 mg/ kg/ day.[8]

Enhancing recruitment through broader international collaboration:

Multicentre RCTs with neonatal outcomes conducted by ANZNN members, funded or co-funded by NHMRC and reported in *The New England Journal of Medicine* in 2001-11[46, 48, 51-53, 59, 60] had a mean recruitment period of 5.4 years, (excluding periods for follow up, analysis and reporting). This may, in part, reflect the limited population of 27 million in Australia and New Zealand.
One strategy to address this issue is through partnership with a well-established overseas network. The Vermont Oxford Network is a voluntary, self-funded 20-year-old quality improvement organisation of health professionals and parents in over 900 neonatal intensive care units, including over 600 in the United States.[61-63] LIFT is the first Australian-led neonatal multicentre RCT to work with US member NICUs in the Vermont Oxford Network, for which Roger Soll is Director of Clinical Trials. Table 3 shows its rates for the adverse outcomes which will be studied in LIFT.

Table 3: Outcomes in 53,448 VLBW infants in Vermont Oxford NICUs, 2010

	Total	**Cases**	**%**
Death in hospital after 48 hours	51,383	4,774	9.3
IVH, grade 3 or 4 before day 28	47,673	4,118	8.6
PVL	49,320	1,584	3.2
Chronic Lung Disease at 36 weeks‡‡	42,777	11,675	27.3
Retinal cryosurgery or laser surgery for ROP	51,854	2,002	3.9
Late onset sepsis (bacterial or fungal) after day 3	51,087	8,109	15.9
NEC	51,861	3,480	6.7

What are the benefits of consumer and community participation?

Australian, UK and US government bodies emphasize the need for strong partnerships between clinicians, consumers, patients and parents.[64-66] These offer many benefits.[67, 68] Parents and consumers will contribute to all stages of the study. They help make information more

understandable and relevant.[69, 70] Their endorsement will enhance recruitment. Their perspectives will assist in communicating results sensitively to participants and the community.[70-73]

Estimating the cost effectiveness of bLF: the importance of a societal perspective

Introduction of new treatments is accelerated by unequivocal evidence that they are cost effective. However, most cost effectiveness studies, in Australia and internationally, seriously undervalue the potential savings –or "opportunity costs" − from preventing morbidity and disability because they capture few or no costs beyond the health system itself.[74] They therefore fail to meet the high standards of evidence required by government agencies, which recommend adopting a societal perspective and including estimates of *indirect costs,* such as lost productivity. The LIFT cost-effectiveness analysis will thus include, in addition to hospital costs, a survey of parental employment and income to capture more completely the potential cost effectiveness and societal benefits of bLF in preventing major morbidity.

2 TRIAL OBJECTIVES

This pragmatic, randomized clinical trial in 1,500 very low birth weight infants (VLBW: <1,500 g)

(I) aims to test the hypotheses that adding bovine lactoferrin (bLF) to feeds improves the following outcomes:-

Primary composite outcome of survival to hospital discharge free from (1) any of three morbidities diagnosed or treated in hospital by 36 weeks: brain injury or late onset sepsis or necrotising enterocolitis (NEC); and free from (2) retinopathy of prematurity (ROP) treated before discharge by local guidelines.

Secondary outcomes: (a) survival to hospital discharge; (b) brain injury to 36 weeks, (c) ROP treated before hospital discharge by local guidelines, (d) late-onset sepsis to 36 weeks, (e) NEC to 36 weeks, (f) time to reach full enteral feeds, (g) number of blood transfusions to 36 weeks, (h) chronic lung disease to 36 weeks, (i) length of hospital stay.

(II) aims to conduct a Cost Effectiveness Analysis of bLF.

(III) aims to evaluate the effect of bLF on survival and developmental outcomes at 24 and 36 months

3 TRIAL DESIGN

3.1 Design

LIFT is a multi-centre, 2-arm, randomised controlled trial.

3.2 Randomisation

Babies will be allocated to bLF or not via an automated centralised randomisation system administered by the NHMRC Clinical Trials Centre. Randomisation will be stratified by site, gender, birth weight (<1000; ≥1000-1499g) and single or multiple birth. Site staff will randomise eligible babies using the centralised system according to the instructions in the Study Manual.

3.3 Endpoints

3.3.1 Primary Composite Endpoint

Survival to hospital discharge:

(1) free from major morbidity at 36 weeks corrected gestational age defined as:

- Brain injury on ultrasound or,
- Necrotising enterocolitis of Grade II or higher or,
- Late onset sepsis; and,

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

3.3.2 Definitions of primary outcome for study

Morbidity Measures

Based on definitions used by the Australian and New Zealand Neonatal Network. [1]

- *Brain injury on ultrasound*
  Grade of 3 and 4 IVH (major intraventricular haemorrhage) seen on ultrasound according to the system of grading defined below:
  1. Subependymal germinal matrix bleed
  2. IVH without ventricular distension
  3. IVH with ventricular distension with blood
  4. Intraparenchymal haemorrhage

  Or echodense intraparenchymal lesions, periventricular leukomalacia, porencephalic cysts or ventriculomegaly (97 percentile plus 4mm), or
- Severe *retinopathy* warranting treatment with laser surgery, cryotherapy or monoclonal antibody therapy according to local guidelines, or
- *Necrotising enterocolitis:* a proven diagnosis with the following 3 criteria:
  1. at least one systemic sign: temperature instability, apnoea, bradycardia or lethargy and at least one intestinal sign: residual of 25% of the previous feed on 2 consecutive occasions, or abdominal distension, or vomiting or faecal blood
  2. profile consistent with definite NEC including at least one of the following: abdominal wall cellulitis and palpable abdominal mass, or pneumatosis intestinalis, or portal vein gas, or a persistent dilated loop on serial X rays, or a surgical or post mortem diagnosis.
  3. warranted treatment for NEC, which included nil by mouth and antibiotics

- *Late onset Sepsis*
  Systemic sepsis is defined as a clinical picture consistent with sepsis, and either a positive bacterial or fungal culture of blood and/or cerebrospinal fluid, or a positive urine culture by sterile collection only. At least one episode of systemic sepsis with initial symptoms from 48 hours after birth.

  General guidelines for identifying positive cultures include:
  Isolation of organisms from one blood culture and, after considering clinical/laboratory evidence, decision made to give antibiotics with therapeutic intent against this organism. Infections with coagulase − negative staphylococci, and other potential contaminants, or group β streptococcal antigen detected in urine should be included only if the baby is considered clinically septic and there is supporting evidence such as raised white cell count or thrombocytopenia. Viral infections must be proven by culture and/ or haematological results consistent with infection. The following must not apply: mixed CNS or other skin flora contaminant; same blood organisms isolated from blood during the previous 14 days – repeat isolate.

3.3.3 Secondary outcomes

1. Survival to hospital discharge
2. Each of the other 4 individual components of the composite primary endpoint
3. Time to full enteral feeds (≥120ml/kg/day for 3 consecutive days)
4. Number of blood transfusions to 36 weeks
5. Chronic lung disease (also known as bronchopulmonary dysplasia) defined as receiving supplemental oxygen or any form of assisted ventilation at 36 weeks for 4 consecutive hours in a 24 hour period
6. Length of hospital stay
7. Financial costs (for cost-effectiveness analysis in Australia only)

3.3.4 Long-Term Outcomes

1. Survival and developmental outcomes at 24 to 36 months (corrected age)

   Major disability is assessed by;
   (i) parent report on the Ages and Stages Questionnaire (ASQ),
   (ii) a modified Short Health Status Questionnaire completed by a medically qualified practitioner documenting either:-
      (a) major developmental delay, including language or speech problems, or
      (b) cerebral palsy with inability to walk unassisted at or after 2 yrs corrected age, 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.
   (iii) Bayley-III Scales of Infant and Toddler Development (Bayley-III) will be collected from all infants where routinely available. A pre-specified sub-cohort of ~20% of survivors within the trial will be used to derive a cut-off score on ASQ equivalent to 2 SDs below the trial norm for cognitive scores on the Bayley-III,

4 SUBJECT POPULATION

4.1 Subject Population

Infants born with a birth weight of less than 1500g and less than or equal to 7 days of age are eligible to join this trial.

4.2 Inclusion criteria

Babies are eligible if
(a) <1500 g birth weight
(b) ≤7 days old and expected to survive
(c) parent gives written informed consent.

4.3 Exclusion criteria

Babies with severe congenital anomalies which are likely to cause death are not eligible.

4.4 Withdrawal criteria

Any family that wishes to withdraw their baby from the trial may do so, without giving a reason and without any change in any other aspect of treatment. Parents of any baby who is withdrawn from the study after randomisation and before or after the intervention is administered, permission will be sought to follow baby's progress and allow collection of outcome data. Parents may choose to withdraw this permission as well. Parents may withdraw their consent for provision of specific information, such as economic data, while continuing to participate in the clinical study.

All such patients will remain in the group to which they were randomly allocated for the purposes of data analysis, which will be conducted by "intention to treat".

5 TREATMENT OF SUBJECTS

5.1 Study Drug Administration

The study is masked, i.e. treatment allocation will be concealed from investigators, clinicians and parents.

A designated person (e.g. bedside nurse, pharmacist, member of milk kitchen staff, or any appropriate staff member) will prepare the milk feed containing either bLF (treatment group) or nothing (control group). This masking procedure avoids use of a placebo, such as sucrose or

maltose, which cannot be guaranteed to be biologically and physiologically inert in preterm or VLBW infants.

Study treatment or control will start as soon as possible after randomisation and continue until 34 weeks corrected gestation or for 2 weeks, whichever is longer, or until discharge home, if earlier.

Infants allocated to the treatment group will receive a once daily dose of lactoferrin in breastmilk or formula milk to a daily dose of up to 250 mg/ kg bLF.

5.2 Concomitant Medications/Treatments

No concomitant medications or treatments are contraindicated. Concomitant medications will not be recorded during the study, except for medications being taken when a SUSAR (suspected unexpected serious adverse reaction) is encountered.

5.3 Compliance

Daily treatment administration or missed doses will be recorded in the study treatment log

5.4 Unblinding

Usually unmasking will only be permitted if knowledge of investigational product is needed for treatment of a Serious Adverse Event. In an emergency, sites shall contact the CTC Co-ordinating Centre to obtain treatment identity. However, discussion with the Chief Investigator or delegate is required prior to unmasking.

5.5 Subject Follow-up

To facilitate good follow up, regular contact after discharge by phone and/or post will be maintained with the families and their nominated relatives and friends to confirm the family's contact details in case of a change of address.

Cost effectiveness outcomes will be measured at one and two years of age. At 24 to 36 months of corrected gestational age, hospitals will arrange for an assessment of developmental delay as outlined in 3.3.4.

Consent will also be sought at recruitment for continuing follow up to 5 years of age.

Subjects in whom study treatment is stopped before the time recommended in the protocol will continue to be invited for follow-up visits, according to the protocol.

If a parent wishes to stop the study visits, they will be asked to allow their baby's ongoing health status to be periodically reviewed either via phone contact with them or by contact with their general practitioner, or by review of their medical records or access to the national mortality registry.

For parents who have been lost to follow-up in Australia, Medicare may be used to provide updated contact information and/or hospitalisations and the national mortality registry may be used to collect mortality information.

6 EFFICACY AND SAFETY

6.1 Assessment of Efficacy

The efficacy of the intervention will be assessed by medical record review for the incidence of death and morbidity before hospital discharge. No specific study visits are required during this period.

Data will be collected for the baby's entire stay in hospital, up until discharge to home or death. Infants transferred to other hospitals prior to discharge home will be tracked by the local trial

coordinator and data about the baby's care in each unit will be collected to ensure that data regarding outcomes are complete.

Developmental outcomes at 24- 36 months and survival will be assessed using the approaches detailed in Section 3.3.4.
Data relating to subsequent health service use and hospitalisations will be collected retrospectively using 1-yr & 2-yr parent questionnaires and a short health questionnaire to be completed by a clinician.

6.2 Assessment of Safety

6.2.1 Definitions

An ADVERSE EVENT (AE) is any untoward medical occurrence in a patient or clinical investigational subject administered a treatment and which does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavourable or unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal investigational product, whether or not considered related to the medicinal product. An adverse event is any adverse change (developing or worsening) from the patient's pre-treatment condition, including intercurrent illness. A SERIOUS ADVERSE EVENT (SAE) is any untoward medical occurrence that at any dose:

- results in death,
- is life-threatening (i.e. the subject is at risk of death at the time of the event),
- requires inpatient hospitalisation or prolongation of existing hospitalisation, or
- results in persistent or significant disability or incapacity or is a congenital anomaly/birth defect.

AEs and SAEs should only be reported if they meet the criteria for a Suspected unexpected serious adverse event (SUSAR: see paragraph 6.2.2 below). SUSARS are reported via eCRFs.

NOTES:

(i) The term "life-threatening" in the definition of "serious" refers to an event in which the patient was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe.
(ii) Important medical events which may not be immediately life-threatening or result in death or hospitalization but which may jeopardize the patient or may require intervention to prevent one of the listed outcomes in the definition above should also be considered serious.

Serious adverse events which may be life threatening are common in very preterm infants, however, the proportion of **unexpected serious adverse events** (in the opinion of the investigators) is expected to be small.

A SUSPECTED UNEXPECTED SERIOUS ADVERSE REACTION (SUSAR) is an SAE that is related to the drug or device and is unexpected [i.e. not listed in the investigator brochure or approved Product Information; or is not listed at the specificity or severity that has been observed; or is not consistent with the risk information described in the Patient Information Sheet and Informed Consent Form or elsewhere in the protocol. (FDA, Safety Reporting Requirements for INDs and BA/BE Studies, draft guidance, September 2010)].

An event is casually related if there is a reasonable possibility that the drug (intervention) caused the AE, i.e. there is evidence to suggest a casual relationship between the drug and the event (FDA, Safety Reporting Requirements for INDs and BA/BE Studies, draft guidance, September 2010).

6.2.2 Reporting of SUSAR (Suspected Unexpected Serious Adverse Reactions)

AEs and SAEs are not required to be reported unless they meet SUSAR criteria. SUSARS are reported via eCRFs.

The investigator is responsible for reporting all SUSARs occurring during the study to the NHMRC Clinical Trials Centre within 1 working day of becoming aware of the event.

LIFT Trial Management Committee and all other Principal Investigators participating in the study will be informed of the SUSAR. The investigator or delegate at each participating institution is responsible for reporting suspected unexpected serious adverse events to their HREC.

Details of the SUSARs will be reviewed by the Independent Data and Safety Monitoring Committee.

6.3 Schedule of Data Collection

Information on in-hospital outcomes will be collected via medical record review from the date of randomisation until hospital discharge. Long-term outcome data, as well as cost information will be collected in the follow-up phase up to 3 years using relevant questionnaires.

	Screening	Randomisation	Baseline	36 weeks	Discharge	Follow-up				
						6 mths	12 mths	18 mths	24 mths	36 mths
Informed Consent	X									
Contact Information	X				X	X		X	X	
Physical Assessments		X	X	X	X					
Outcome events				X	X					
Phone contact (Vital Status)						X	X	X	X	X
Parental Labour Force Participation Questionnaire			X				X		X	X
Child Hospital Use							X		X	X
Ages and Stages Questionnaire									X[#]	X[#]
Short Health Questionnaire									X[#]	X[#]
Bayley III**									X[#]	X[#]

***in a random sub-cohort of ~20% of survivors within the trial and where routinely performed.*
[#] assessments will be collected at either 24 or 36 months, in line with routine follow up scheduling for each hospital.

7 INVESTIGATIONAL PRODUCT

7.1 Investigational Product

The investigational product is bovine Lactoferrin (bLF).

7.2 Supply of Investigational Product

Lactoferrin is supplied by AUSTRALIA'S OWN PTY LTD. This product is listed on the Australian Therapeutics Goods Register.

7.3 Drug Accountability

The Pharmacy Department (or relevant department such as milk kitchen) at the participating institutions will maintain a record of feeds supplemented with study intervention for each patient.

8 STATISTICS

8.1 Sample Size

A sample size of 1,500 infants has 85% power at the two-sided 5% significance level to detect a difference in the proportion meeting the primary outcome assuming the true probability is 74% in controls and 80.5% in infants having bLF. The power of the trial remains above 80% even if non-adherence to randomized treatment occurs in 5% of participants. A non-adherence rate <5% is likely based on our previous trial[8]. The estimated proportion meeting the primary outcome in the control arm is informed by pre-trial estimates (see Table 3 and Table 4), blinded (pooled) review of accumulating trial data (most recently undertaken in December 2016), and the anticipated beneficial effects of the growing use of probiotics and downward trend in rates of sepsis [76, 77].

8.2 Statistical Analysis

A statistical analysis plan will be prepared and finalised prior to unblinding the data. All randomised subjects will be eligible for inclusion in efficacy analyses in accordance with the intention-to-treat analysis principle. Subjects will be analysed according to the regimen they actually received for comparisons on SUSAR rates.

The primary analysis will be a comparison between treatment groups on the proportion experiencing the primary outcome using a chi-squared statistic that accommodates possible correlation of data between siblings from multiple births. Other binary secondary outcomes will be analysed using the same method, whilst comparable approaches applicable to continuous data will be applied as required. Estimates of the treatment effect adjusted for baseline characteristics will be calculated in sensitivity analyses using the relevant linear modelling approach. These modelling techniques will also be used to identify clinically important prognostic factors and to perform tests of heterogeneity in the subgroup analyses.

Hypothesis tests will be undertaken at the two-sided 5% level of significance. P-values from secondary analyses that are unadjusted for multiple comparisons will be interpreted in proper context.

Subgroup analyses: Consistency of the treatment effect on the primary endpoint will be evaluated across the following subgroups: (i) birthweight <1000 g and 1000-1499 g; (ii) randomised ≤72 hr and >72 hr from birth; (iii) those who received and did not receive probiotics by 36 weeks corrected gestation; (iv) ≤ 28 weeks and >28 weeks gestation at birth.

8.3 Cost Effectiveness of BLF

The incremental cost effectiveness ratio (ICER) for BLF will be calculated directly from trial data and defined as cost per life saved without morbidity. Each hospital admission for child birth will be assigned an Australian-Refined Diagnosis Related Group (AR-DRG) and an associated cost weight based on the type of delivery and the level of complications and co-morbidities present. In addition, each neonatal admission will be classified into an AR-DRG based on the collection of several key data items, including the admission weight of the neonate, whether a significant operating room procedure occurred, the level of major complications that occurred and whether a

death or transfer occurred within 5 days of admission. Costs for inpatient care will be estimated using the most recent AR-DRG costs weights available at the time of analysis.

Data on non-hospital health care use are available for Australian patients through a central registry. Australian patients will be invited to give their consent for the NHMRC Clinical Trials Centre to obtain their Medicare claim data from the federal government via the Health Insurance Commission. The Medicare system provides reimbursement for pharmaceutical benefits schedule (PBS) and medical benefits schedule (MBS) health care costs incurred by individuals within Australia. The claim data kept by the Health Insurance Commission therefore provides a reliable estimate for the medication and medical services patients receive outside of the hospital setting. In addition, Australian parents will be surveyed at baseline on their current employment, income, savings and plans for work after childbirth and then yearly to estimate the impact child morbidity, on workforce outcomes.

9 STUDY STRUCTURE

The study will be coordinated by the NHMRC Clinical Trials Centre.

9.1 Trial Management Committee

The trial will be managed by a Trial Management Committee consisting of local and international collaborators. A Trial Executive Committee (TEC) may be selected from the TMC in order to expedite decision-making and will be led by the Study Chair. The TEC is a subset of the TMC which meets more regularly on key scientific and/or operational issues impacting on study conduct. The NHMRC Clinical Trials Centre, as the study coordinating centre will support this and other study committees and processes.

9.2 Independent Data and Safety Monitoring Committee

An Independent Data and Safety Monitoring Committee (IDSMC) will monitor the progress of the study patient safety, and appropriateness of study design. It will review interim data and other emerging evidence, including relevant RCTs and overviews of RCTs. The IDSMC will advise the TMC if in their view there is proof beyond reasonable doubt of net clinical benefit or harm, for all infants or for a subset of infants, that might reasonably be expected to influence the management of many clinicians. Data on key study outcomes will be reviewed when outcomes are available for the first N=550 and thereafter every 12 months, or more frequently if requested by the IDSMC. A charter will be prepared detailing the role and responsibility of the IDSMC as well as operational, decision making, and reporting processes.

10 ADMINISTRATIVE ASPECTS

10.1 Ethics and regulatory compliance

This study will be conducted according to the Note for Guidance on Good Clinical Practice (CPMP/ICH/135/95) annotated with TGA comments (Therapeutic Goods Administration DSEB July 2000) and in compliance with applicable laws and regulations. The study will be performed in accordance with the NHMRC Statement on Ethical Conduct in Research Involving Humans (© Commonwealth of Australia 2007) and the principles laid down by the World Medical Assembly in the Declaration of Helsinki 2008. In addition, overseas collaborators will need to comply with their requisite local regulations. The investigator shall comply with the protocol, except when a protocol deviation is required to eliminate immediate hazard to a subject. In this circumstance the CTC, Chief Investigator and HREC must be advised immediately. The trial will be registered with the Australian and New Zealand Clinical Trials Registry.

10.2 Confidentiality

The study will be conducted in accordance with applicable Privacy Acts and Regulations. All data generated in this study will remain confidential. All information will be stored securely at the NHMRC Clinical Trials Centre, University of Sydney and will only be available to staff directly involved with the study.

10.3 Protocol amendments

Changes and amendments to the protocol can only be made by the Trial Management Committee. Approval of amendments by the Institutional HREC is required prior to their implementation. In some instances, an amendment may require a change to a consent form. The Investigator must receive approval/advice of the revised consent form prior to implementation of the change. In addition, changes to the Case Report Forms (CRFs), if required, will be incorporated in the amendment.

The investigator should not implement any changes to, or deviations from, the protocol except where necessary to eliminate immediate hazard(s) to trial subject(s).

10.4 Data Handling and Record Keeping

Trial data will be recorded on the CRFs (case report forms) and (e-)CRFs (electronic case report forms) provided. All required data entry fields will be completed. Data corrections will be done according to the instructions provided. The investigator will be asked to confirm the accuracy of completed CRFs by signing key CRFs and/or study books as indicated.

Source documents pertaining to the trial must be maintained by investigational sites. Source documents may include a subject's medical records, hospital charts, clinic charts, the investigator's subject study files, as well as the results of diagnostic tests such as X-rays, laboratory tests, and electrocardiograms. The investigator's copy of the case report forms serves as part of the investigator's record of a subject's study-related data.

The following information should be entered into the subject's medical record:

a. Baby's name, contact information and protocol identification.
b. The date that the baby entered the study, and subject number.
c. A statement that informed consent was obtained (including the date).
d. Relevant medical history
e. Results of key trial parameters.
f. Occurrence of any SUSARs or outcome events.
g. The date the baby exited the study, and a notation as to whether the subject completed the study or reason for discontinuation.

All study-related documentation will be maintained for 23 years following completion of the study.

10.5 Study Monitoring

Data from this study will be monitored by Clinical Trials Program staff from the NHMRC Clinical Trials Centre (CTC). Monitoring will include centralized review of CRFs and other study documents for protocol compliance, data accuracy and completeness. Monitoring may include monitoring visits to investigational sites for source data verification, review of the investigator's site file and drug handling records. By signing the informed consent form, the parent gives authorized CTC staff direct access to their child's medical records and the study data.

10.6 Audit and Inspection

This study may be subject to audit or inspection by representatives of the CTC or representatives of relevant regulatory bodies.

10.7 Clinical Study Report

The data will be cleaned and statistical analysis will be conducted by the NHMRC CTC. A Clinical Study Report will be issued which may form the basis of (a) manuscript(s) intended for publication.

10.8 Publication Policy

The Trial Management Committee will appoint a Writing Committee to draft manuscripts based on the trial data. Manuscripts will be submitted to peer-reviewed journal(s). The main publication will be the report of the full trial results based on the main protocol using the study group name, with subsequent publications of data subsets. The Writing Committee will develop a publication plan, including authorship, target journals and expected dates of publication.

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