A multi-centre randomised placebo-controlled trial of prophylactic enteral lactoferrin supplementation to prevent late-onset invasive infection in very preterm infants

Protocol

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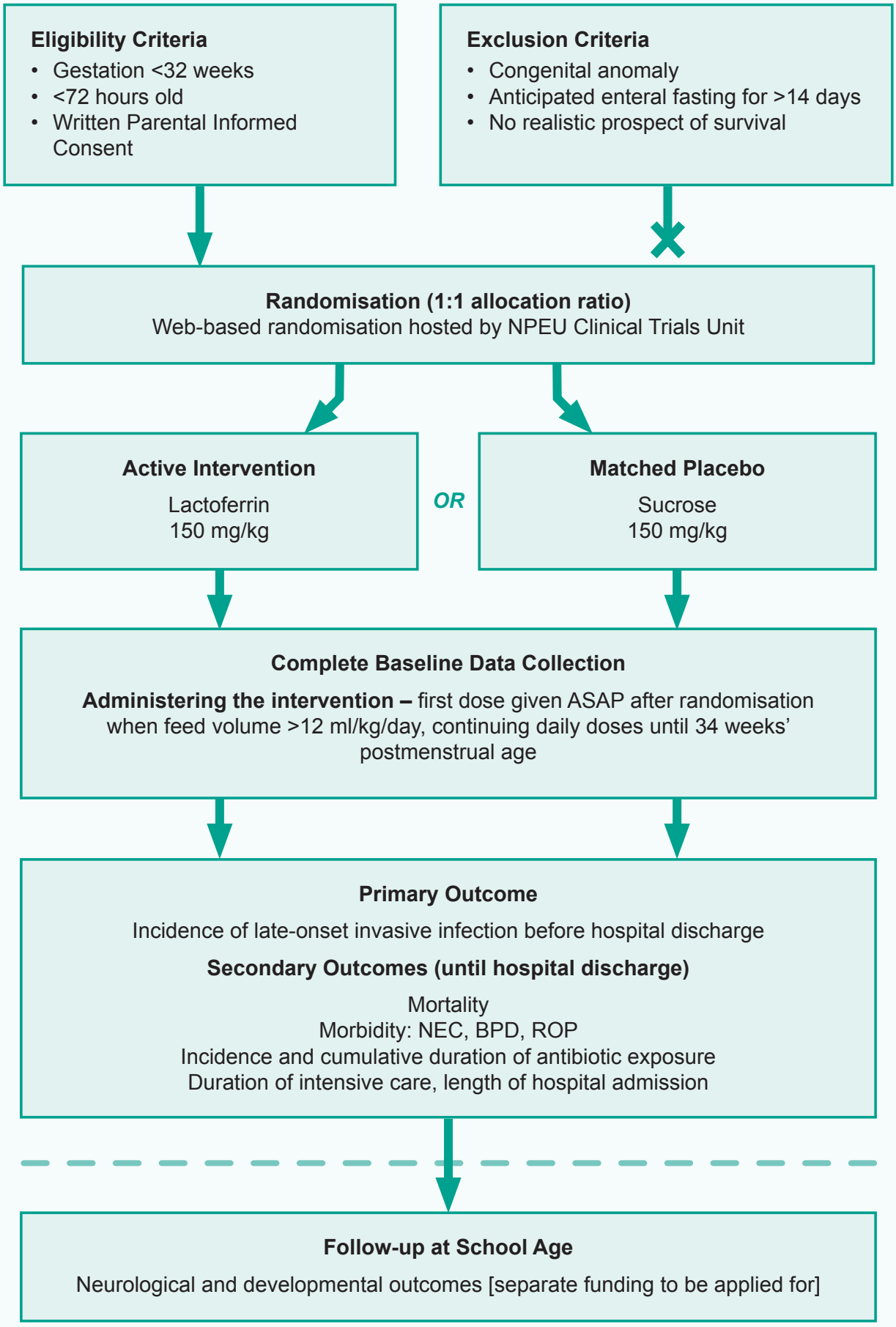
Contents

1. Trial Summary

Study Title	ELFIN – a multi-centre randomised placebo-controlled trial of prophylactic enteral lactoferrin supplementation to prevent late-onset invasive infection in very preterm infants
Internal ref. no.	ELFIN01
Clinical Phase	Phase III
Trial Design	Multi-centre, blinded, placebo controlled randomised controlled trial (RCT). An initial nine month pilot phase will be followed by a three year main recruitment phase.
Trial Participants	Very preterm infants (<32 weeks)
Inclusion Criteria	Infants will be eligible to participate if: • Gestational age at birth is less than 32 weeks • Less than 72 hours old • Written informed parental consent is obtained If infants are receiving antibiotic treatment for suspected or confirmed infection, they are still eligible for recruitment
Exclusion Criteria	• Infants with a severe congenital anomaly • Anticipated enteral fasting of more than 14 days • Infants who, in the opinion of the treating clinician, have no realistic prospect of survival
Planned Sample Size	2,200 (including pilot)
Follow-up Duration	If a statistically significant and clinically important effect on the primary outcome is detected, further funding will be sought and approval to assess the impact of lactoferrin supplementation on rates of adverse neuro-developmental outcomes in participating infants when they are school age
Planned Trial Period	57 months (including pilot)
Primary Objective	To test the efficacy of the enteral administration of 150 mg/kg/day of bovine lactoferrin in reducing the incidence of microbiologically-confirmed or clinically suspected late-onset infection (defined as more than 72 hours after birth) from trial entry until hospital discharge in a population of very preterm infants.
Primary Outcome	The incidence of microbiologically-confirmed or clinically suspected late onset-infection from trial entry until hospital discharge
Secondary Objectives	In a population of very preterm infants, did lactoferrin supplementation reduce any of the following?: • All-cause mortality prior to hospital discharge • NEC: Bell's stage II or III • Severe ROP treated medically or surgically • BPD: infant is still receiving mechanical ventilator support or supplemental oxygen at 36 weeks' postmenstrual age • A composite of invasive infection, major morbidity (NEC, ROP, or BPD as defined above) and mortality • Total number of days of administration of antibiotics per infant from 72 hours until death or discharge from hospital • Total number of days of administration of antifungal agents per infant • Total length of stay until discharge home • Length of stay in (i) intensive care, (ii) high dependency care, (iii) special care
IMP	Bovine lactoferrin
Form	Powder and solvent for oral solution
Dose	150 mg/kg/day (up to a maximum of 300 mg/day)
Route	Enteral

2. Trial Flow Diagram: Lactoferrin supplementation for very preterm infants



3. Abbreviations

ADR	Adverse Drug Reaction
AE	Adverse Event
AR	Adverse Reaction
ARR	Absolute Risk Reduction
BPD	Bronchopulmonary Dysplasia
CER	Control Event Rate
CI	Chief Investigator
CLD	Chronic Lung Disease
CSF	Cerebro-spinal Fluid
CTA	Clinical Trials Authorisation
CTRG	Clinical Trials & Research Governance team, University of Oxford
DCF	Data Collection Form
DMC	Data Monitoring Committee
DSUR	Development Safety Update Report
EBM	Expressed Breast Milk
ELBW	Extremely Low Birth Weight (defined as < 1,000 g)
ELFIN	Enteral Lactoferrin in Neonates
GCP	Good Clinical Practice
GMP	Good Manufacturing Practice
GP	General Practitioner
HTA	Health Technology Assessment
IB	Investigators Brochure
ICER	Incremental Cost-Effectiveness Ratio
ICF	Informed Consent Form
ICH	International Conference of Harmonisation
IMP	Investigational Medicinal Product
IRAS	Integrated Research Application System
LGG	Lactobacillus Rhamnosus GG
LRN	Local Research Nurse
MHRA	Medicines and Healthcare products Regulatory Agency
NEC	Necrotising Enterocolitis
NHS	National Health Service
NIHR	National Institute for Health Research
NPEU CTU	National Perinatal Epidemiology Unit Clinical Trials Unit
NRES	National Research Ethics Service
NSAID	Non-Steroidal Anti-inflammatory Drug
PI	Principal Investigator

PIL	Participant/Patient Information Leaflet
PMG	Project Management Group
R&D	NHS Trust R&D Department
RCT	Randomised Controlled Trial
REC	Research Ethics Committee
ROP	Retinopathy of Prematurity
RR	Relative Risk
SAE	Serious Adverse Event
SAR	Serious Adverse Reaction
SMPC	Summary of Medicinal Product Characteristics
SOP	Standard Operating Procedure
SUSAR	Suspected Unexpected Serious Adverse Reaction
TMF	Trial Master File
TSC	Trial Steering Committee
VLBW	Very Low Birth Weight (defined as birth weight < 1,500 g)

4. Introduction

4.1 Summary of Trial Design

This is a multi-centre, placebo-controlled randomised controlled trial (RCT) assessing the effect of prophylactic enteral lactoferrin supplementation on morbidity and mortality in very preterm (gestational age at birth < 32 weeks) infants. If feasibility is shown during an initial pilot phase of nine months duration, a total of 2,200 infants will be recruited to participate in the trial over a four year period.

4.2 Epidemiology of Late-onset Infection in Very Preterm Infants

Late-onset invasive infection (occurring more than 72 hours after birth) is the most common serious complication associated with intensive care for newborn infants. The incidence is generally estimated to be higher than 20% in very preterm infants reflecting the level and duration of their exposure to invasive procedures and intensive care[1-3]. Very preterm infants with late-onset invasive infection often have a need for intensive care and mechanical ventilation. They are also at higher risk of mortality and a range of important morbidities including necrotising enterocolitis (NEC), retinopathy of prematurity (ROP), and bronchopulmonary dysplasia (BPD)[1,2,5,7-10]. These higher rates of mortality and serious morbidity are usually associated with Gram-negative bacterial or fungal infections. Coagulase-negative staphylococcal infection, while more common, being responsible for about 50% of infections[4], is generally associated with a more benign clinical course. Meningitis and other deep-seated infections are rare and associated mortality is much lower. However, even a low grade coagulase-negative staphylococcal bloodstream infection may generate inflammatory cascades which are associated with both acute morbidity (metabolic, respiratory or thermal instability, thrombocytopenia) and long-term white matter and other brain damage that may result in adverse neuro-developmental outcomes[11].

As a consequence of associated morbidities, very preterm infants with invasive infection spend about 20 more days in hospital than similar infants without infection[2]. Late-onset infection therefore has major consequences for perinatal health care, service management, delivery and costs.

4.3 Diagnosis, Treatment and Prevention of Late-onset Invasive Infection

Clinical signs and laboratory markers are unreliable predictors of true invasive infection, especially in very preterm infants[12,13]. A policy of early empirical treatment of suspected infection is usually implemented. However, most neonates who are treated as a result of “sepsis evaluation” do not have infection subsequently confirmed[14]. This results in unnecessary exposure to antibiotics which not only subjects very preterm infants to more interventions, but may drive the emergence of antibiotic-resistant pathogens in the neonatal nursery[15,16]. While generic infection control measures such as hand-washing and intravascular catheter care ‘bundles’ have helped to reduce late-onset invasive infection, benchmarking and quality improvement studies in neonatal networks have indicated that there is a need for measures to further reduce the incidence[17,18]. Given this burden of mortality, acute morbidity, long term morbidity and costs to families and health services, there is a need to develop innovative strategies to prevent late-onset invasive infection in very preterm or very low birthweight (VLBW) infants.

4.4 Lactoferrin

Lactoferrin, a member of the transferrin family of iron-binding glycoproteins, is a key component of the mammalian innate response to infection. It is the major whey protein in human colostrum, present at a concentration of about 6 mg/ml and in mature breast milk at a concentration of about 1 mg/ml. It is also present in mammalian tears, saliva, cerebro-spinal fluid (CSF), and other secretions. However, very preterm infants ingest little or no milk during the early neonatal period and thus have low lactoferrin intake. This may be further exacerbated by delay in establishing enteral feeding[19,20].

Lactoferrin has broad microbiocidal activity by mechanisms such as cell membrane disruption, iron sequestration, inhibition of microbial adhesion to host cells, and prevention of biofilm formation[21, 22]. Development of resistance to lactoferrin is improbable as it would require multiple simultaneous mutations. Lactoferrin remains a potent inhibitor of viruses, bacteria, fungi, and protozoa after millions of years of mammalian evolution[23-25].

Lactoferrin also has prebiotic properties, creating an enteric environment promoting the growth of beneficial bacteria and reducing colonisation with pathogenic species. It also has direct intestinal immunomodulatory and anti-inflammatory actions mediated by modulating cytokine expression, mobilising leucocytes into the circulation, and activating T-lymphocytes[26-29]. At high concentrations, as in colostrum, lactoferrin enhances proliferation of enterocytes and closure of enteric gap junctions. At lower concentrations, lactoferrin stimulates differentiation of enterocytes and expression of intestinal digestive enzymes[30]. Lactoferrin suppresses free radical activity when iron is added to milk, suggesting that it may have further anti-inflammatory actions that could modulate the pathogenesis of diseases linked with free radical generation such as NEC, ROP, and BPD[31].

4.5 Bovine Lactoferrin

Bovine lactoferrin is >70% homologous with human lactoferrin but has higher antimicrobial activity. It is inexpensive compared with human or recombinant lactoferrin and is available commercially as a food supplement in a stable powder form[32,33]. The affinity of bovine lactoferrin for the human small intestine lactoferrin receptor is low and intact lactoferrin and digested fragments (lactoferricins), which also have high microbicidal activity, are excreted in stools. Bovine lactoferrin has been a component of the human infant diet for thousands of years and is registered as 'Generally Recognised As Safe' by the US Federal Drug Administration with no reports of human toxicity. The 'no-observed-adverse-effect level' is more than 2g/kg/day in rodents[35].

4.6 Administration of Lactoferrin

Previous trials have given 100 mg of lactoferrin mixed in 2 ml or less of breast milk or formula making it feasible to administer to most very preterm infants during the first week after birth[35]. The osmolality of 100 mg of lactoferrin in 2 ml of breast milk or formula is less than 343 mOsm/kg making it acceptable for enteral administration. In this trial, 375 mg of lactoferrin will be dissolved in 5 ml of total fluid volume, 4 ml water plus 1 ml breast milk, giving a concentration of 75 mg/ml. Infants can thus achieve an enteral intake of 150 mg/kg/day of lactoferrin (to a maximum of 300 mg) soon after starting milk feeds.

4.7 Reports of Existing Randomised Controlled Trial of Lactoferrin Supplementation in Very Preterm Infants

The Cochrane review of prophylactic lactoferrin supplementation in very preterm infants identifies only one relevant randomised controlled trial[36,37]. This was conducted in 11 Italian neonatal centres in 2007 and 2008. A total of 472 infants participated in this three-arm trial. The investigators reported that enteral supplementation either with bovine lactoferrin or with lactoferrin plus the probiotic Lactobacillus rhamnosus GG (LGG) reduced the incidence of late-onset invasive infection by approximately two-thirds compared with placebo:

Group	Incidence of late onset invasive infection (%)	Relative risk [95% confidence interval]
Placebo	17.3	-
Lactoferrin only	5.9	0.34 [0.17 to 0.70]
Lactoferrin plus LGG	4.6	0.27 [0.12 to 0.60]

The effect size was similar regardless of whether infants were fed predominantly with human or formula milk. In addition, the incidence of severe ROP was significantly reduced in the lactoferrin only group [3.9% versus 11.3% in controls], as was the incidence of NEC (≥ Stage 2) in both the lactoferrin only and the lactoferrin plus LGG groups (2% lactoferrin only, versus 0% lactoferrin plus LGG, versus 6% in controls). No evidence of an effect on all-cause mortality or BPD was found. Longer term neuro-developmental outcomes were not reported. No adverse effects or intolerance of the treatment were reported.

4.8 Justification for a Trial

The Italian investigators and the Cochrane reviewers concluded that the available evidence remains insufficient to support a change in practice and that the effect of lactoferrin supplementation on late-onset invasive infection, morbidity, and mortality in very preterm infants "needs to be confirmed in well-designed, adequately-powered, multi-centre [RCTs]"[36,37]. The accompanying editorial stated that the results "make further research on lactoferrin a priority, [and that] future research should be directed at confirming the safety and efficacy of lactoferrin [and that] additional outcomes including hospital length of stay and costs should be studied"[38].

There are several justifications for these conclusions:

1. The effect on reducing invasive infection rates detected in the Italian RCT was strong for the extremely low birth weight (ELBW: < 1,000 g) subgroup of infants, being 11.3% for lactoferrin only versus 36.7% for placebo, but a statistically significant effect was not found for infants weighing 1,000–1,500 g. This is consistent with a lower incidence of invasive infection in larger infants and a diminishing dose-response effect reflecting the fixed dosage of 100 mg/day. Infants in the ELBW group also received lactoferrin for longer: 6 weeks, compared to 4 weeks for infants weighing 1,000–1,500 g
2. It is possible that lactoferrin supplementation may have additional beneficial effects if given at daily doses closer to levels ingested by enterally-fed term infants (100–200 mg/kg/day) and for all infants until they reach a postmenstrual age of 34 weeks which covers the periods of highest exposure to infection risk[3].
3. Determining effectiveness and cost-effectiveness to allow comparison with other infection control interventions in this population is essential to justify the prophylactic use of this intervention in all very preterm infants (1–2% of all newborn infants).
4. Although the Italian setting of the existing RCT is broadly similar to the UK, there are important differences in clinical practice, particularly in infection control policies and in antibiotic prescribing and stewardship, that limit generalisability of the results of this trial.
5. The incidence of invasive fungal infection in the control group of participants in the Italian trial was 5.4% overall and 10% in the ELBW subgroup; this is five-fold higher than the average incidence in UK neonatal units[7]. In the Italian trial, a substantial proportion of the overall effect on reducing late-onset invasive infection was due to the effect on reducing fungal infection and there was not a statistically significant effect on the incidence of bacterial infection.

4.9 How this Trial Addresses these Issues

Late-onset invasive infection has been pre-specified as the primary outcome but the effects on mortality, other major morbidity, antibiotic usage, and duration of hospital stay have been specified as secondary outcomes. Evidence of effects on these outcomes could strengthen the case for adoption of prophylactic lactoferrin as a "standard of care". The trial will be powered to assess meaningful effects on the more common of these outcomes and it will form part of prospective meta-analysis collaboration with similar trials planned in Australasia and North America. The meta-analysis will allow detection of modest but important effects on the less frequent outcomes[39].

5. Trial Objectives

5.1 Primary Objective

To test the efficacy of the enteral administration of 150 mg/kg/day of bovine lactoferrin in reducing the incidence of **microbiologically-confirmed or clinically suspected late-onset infection** (defined as more than 72 hours after birth) from trial entry until hospital discharge in a population of very preterm infants.

5.2 Secondary Objectives

To determine, in a population of very preterm infants, whether lactoferrin supplementation can reduce:

- All-cause mortality prior to hospital discharge
- NEC: Bell's stage II or III
- Severe ROP treated medically or surgically
- BPD: where the infant is still receiving mechanical ventilator support or supplemental oxygen at 36 weeks' postmenstrual age
- A composite of invasive infection, major morbidity (NEC, ROP, or BPD as defined above), and mortality
- Total number of days of administration of antibiotics per infant from 72 hours until death or discharge from hospital
- Total number of days of administration of antifungal agents per infant
- Total length of stay until discharge home
- Length of stay in (i) intensive care, (ii) high dependency care, (iii) special care

6. Trial Design

6.1 Summary

A multi-centre placebo-controlled RCT with prospective economic evaluation undertaken in 30 neonatal units in the UK. An initial nine month pilot phase will be followed by a three year main recruitment phase seeking to recruit an overall total of 2,200 infants.

6.2 Inclusion Criteria

Infants will be eligible to participate if:

- Gestational age at birth is less than 32 weeks
- Less than 72 hours old
- Written informed parental consent is obtained

If infants are receiving antibiotic treatment for suspected or confirmed infection, they are still eligible for recruitment.

6.3 Exclusion Criteria

- Infants with a severe congenital anomaly
- Anticipated enteral fasting of more than 14 days
- Infants who, in the opinion of the treating clinician, have no realistic prospect of survival

6.4 Setting

Neonatal units in the United Kingdom caring for very preterm infants. It is possible for an infant to participate in more than one clinical trial, depending on the interventions being given. Trials being run simultaneously in any units will be discussed by the Chief Investigators (CIs) or their delegated representative(s) to agree whether or not joint recruitment is acceptable to both parties. Individual circumstances will also be reviewed if needed on a case-by-case basis, in consultation with the CI of each trial.

ELFIN has been designed to run alongside SIFT (Speed of Increasing Milk Feeds Trial) so that infants may be enroled in both trials. The two trials share similar procedures and, in some cases, joint data collection forms and other documentation.

6.5 Primary Outcome

The primary outcome will be the incidence of new episodes of **the incidence of microbiologically-confirmed or clinically suspected late onset-infection** (defined as more than 72 hours after birth) from trial entry until hospital discharge (see Box 1 and Box 2 for definitions).

Box 1: Definition of microbiologically-confirmed late-onset invasive infection

A modified version of the UK Neonatal Infection Surveillance Network case-definition will be used[3]:

Microbiological culture from blood or CSF sampled aseptically more than 72 hours after birth of any of the following:

- potentially pathogenic bacteria (including coagulase-negative Staphylococci species but excluding probable skin contaminants such as diphtheroids, micrococci, propionibacteria or a mixed flora)

- fungi

AND

Treatment for 5 or more days with intravenous antibiotics after the above investigation was undertaken. If the infant died, was discharged, or was transferred prior to the completion of 5 days of intravenous antibiotics, this condition would still be met if the intention was to treat for 5 or more days.

There is no need to report urinary tract infection unless there is also a positive blood culture.

Box 2: Definition of clinically suspected late-onset invasive infection

This is adapted from the European Medicines Agency consensus criteria and the predictive model[40,41]:

Either:

- Absence of positive microbiological culture, OR

- culture of a mixed microbial flora or of likely skin contaminants (diphtheroids, micrococci, propionibacteria) only

AND

Clinician intent to administer antibiotic treatment or intravenous antifungals for 5 or more days (excluding antimicrobial prophylaxis) for an infant who demonstrates 3 or more of the following clinical or laboratory features of invasive infection:

- increase in oxygen requirement or ventilatory support
- increase in frequency of episodes of bradycardia or apnoea
- temperature instability
- ileus or enteral feeds intolerance and/or abdominal distention
- reduced urine output to <1 ml/kg/hour
- impaired peripheral perfusion (capillary refill time >3 seconds, skin mottling or core-peripheral temperature gap >2°C)
- hypotension (clinician defined as needing volume or inotrope support)
- 'irritability, lethargy or hypotonia' (clinician-defined)
- increase in serum C-reactive protein levels to > 15 mg/l or procalcitonin ≥ 2 ng/ml
- white blood cells count < 4 or > 20 × 10^9 cells/l or platelet count < 100 × 10^9/l
- glucose intolerance (blood glucose < 40 mg/dl or > 180 mg/dl)
- metabolic acidosis (base excess (BE) < -10 mmol/l or lactate > 2 mmol/l

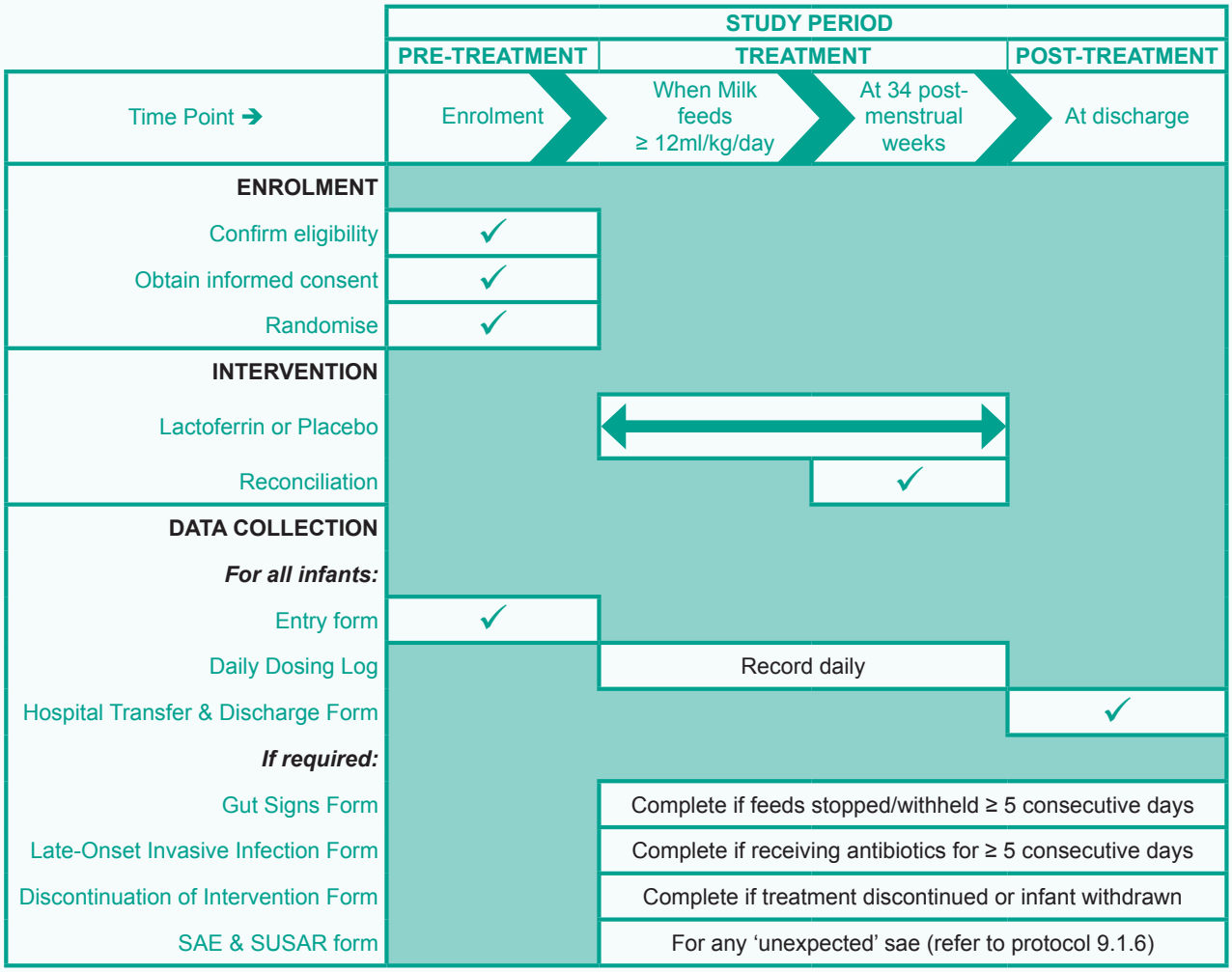
6.6 Secondary Outcomes

In a population of very preterm infants, did lactoferrin supplementation reduce any of the following?:

- All-cause mortality prior to hospital discharge
- NEC: Bell's stage II or III ([42] see Appendix 1)
- Severe ROP treated medically or surgically[43]
- BPD: where the infant is still receiving mechanical ventilator support or supplemental oxygen at 36 weeks' postmenstrual age[44]
- A composite of invasive infection, major morbidity (NEC, ROP, or BPD as defined above), and mortality
- Total number of days of administration of antibiotics administered per infant from 72 hours until death or discharge from hospital
- Total number of days of administration of antifungal agents per infant
- Total length of stay until discharge home
- Length of stay in (i) intensive care, (ii) high dependency care, (iii) special care ([45] see Appendix 2)

7. Trial Procedures

7.1 Participant timeline



7.2 Screening and Eligibility Assessment

Potential participants meeting the eligibility criteria will be identified by the direct healthcare team.

7.3 Informed Consent

Written consent will be sought from the parents only after they have been given a full verbal and written (via the parent information leaflet [PIL]) explanation of the trial. Parents who do not speak English will only be approached if an adult interpreter is available. Relatives may not interpret.

Informing potential trial participants' parents of possible benefits and known risks will occur as a staged process[46]. If it is likely that the expected infant may be eligible to participate in the trial, the parent information leaflet and preliminary verbal information will be offered prior to birth. Further verbal information will be provided after birth as it will to the parents of infants not identified before birth. This will be available both at participating centres and at local hospitals that routinely refer expectant mothers and/or infants into the participating centres.

Written informed parental consent will be obtained by means of dated parental signature and the signature of the person who obtained informed consent; this will be the Principal Investigator (PI) or healthcare professional with delegated authority. A copy of the signed informed consent form (ICF) will be given to the parent(s). A further copy will be retained in the infant's medical notes, a copy will be retained by the PI, and the original will be sent to the NPEU CTU.

At all stages it will be made clear to the parents that they remain free to withdraw their infant from the trial at any time without the need to provide any reason or explanation. Parents will be made aware that this decision has no impact on any aspects of their infant's continuing care.

7.4 Enrolment

After informed consent has been given, the Entry Form should be completed. Information recorded on the Entry Form will be entered onto the randomisation website. Infants will be considered to have been enrolled once they have been given a study number and have been allocated a treatment pack number by the randomisation facility.

7.5 Remuneration

Neither trial participants nor their parents will be given any financial or material incentive or compensation to take part in this trial.

7.6 Randomisation

Randomisation of participants to receive either lactoferrin or sucrose placebo will be managed via a secure web-based randomisation facility hosted by the National Perinatal Epidemiology Unit Clinical Trials Unit (University of Oxford) with telephone back-up available at all times (365 days per year). To confirm eligibility, investigators will need to supply gestational age, sex and time of birth, and to proceed, confirm that signed informed consent forms are available. The randomisation program will use a minimisation algorithm to ensure balance between the groups with respect to the collaborating hospital, sex, multiple births and gestational age at birth. Twins (or higher order multiple births) will be randomised individually.

The Senior Trials Programmer at the NPEU CTU will write the randomisation program and hold the code. If necessary, the code may be broken for a single participant at the request of the Chief Investigator, or their delegated Deputy, by a designated member of staff at the NPEU CTU. See section 7.11 for the procedure for unblinding.

7.7 Trial Interventions

There are no interventions over and above normal clinical care, with the exception of the trial medication. All aspects of care, including the timing of the commencement of enteral feeds and the type of feed used, will be as per local policy, practice, and discretion.

Throughout the trial, participating infants may be prescribed any concomitant medications deemed necessary to provide appropriate supportive care.

7.8 Trial Medication

Trial participants will receive either 150 mg/kg/day (up to a maximum of 300 mg) of lactoferrin or sucrose placebo, as determined by the randomisation, prepared in sterile water (4 ml) plus expressed breast or formula milk (1 ml). Treatment will be normally given once daily by naso/oro-gastric tube or orally once feed volume is equal to or greater than 12 ml/kg/day, until 34 weeks' postmenstrual age. Some small infants may have the dose split at the discretion of the caring physician. A maximum of 70 days of treatment will be given. Distribution and preparation of the trial medication is described in Appendix 3.

All other aspects of care will remain at the discretion of the responsible neonatal team.

7.9 Stopping Trial Medications

Trial medication may be stopped temporarily; missed doses will not necessitate the removal of an infant from the trial. Data will continue to be collected as per protocol if the trial medication is stopped temporarily or permanently in order to facilitate an unbiased treatment comparison, i.e. an intention-to-treat analysis.

7.10 Allocation Concealment and Blinding

Participating infants will be randomly allocated to an individual trial number corresponding to a numbered box containing either the study drug or placebo. Parents, clinicians, investigators, and outcomes assessors will all be unaware of the allocated treatment groups.

7.11 Procedure for Unblinding

In the event of emergency, a participant may be unblinded by contacting the NPEU CTU during working hours, or by calling the helpline out of hours. Contact numbers will be listed on data collection forms and in the Investigator Site File. Details of the person requesting unblinding, and the reason for that request, will be recorded. Wherever possible, the Chief Investigator or their designee must approve the unblinding.

Clinicians requesting emergency unblinding must be satisfied that it is a genuine emergency and that knowledge of the treatment allocation (either lactoferrin or sucrose placebo) is needed to guide the appropriate clinical management of the participant. In some cases this may be achieved without unblinding by treating the participant as if they have received lactoferrin.

As it is best practice to not unblind participants until any follow up is completed, all other requests for unblinding must be made in writing to the NPEU CTU.

7.12 Trial Assessments

No additional blood samples or investigations are required for this trial beyond what would be considered standard care.

7.13 Data Collection

All of the outcome data for this trial are routinely recorded clinical items that can be obtained from the clinical notes or local microbiological laboratory records. Information will be collected using the following DCFs:

Trial Entry Form	The entry form contains sections to be completed before, during and after randomisation, and collects the infant's baseline characteristics
Daily Dosing Log	To be completed daily during the treatment period (once the infant receives milk feeds of 12 ml/kg/day until 34 weeks' postmenstrual age) to document the administration of lactoferrin or placebo, type of milk given, and use of antibiotic and antifungal drugs
Late-Onset Invasive Infection Form	To report each episode of microbiologically-confirmed or clinically-suspected late-onset invasive infection
Gut Signs Form	Complete this form whenever an infant has received at least 5 days of treatment for gut signs, if they are transferred with gut signs, or if they have died from gut signs
Hospital Transfer & Discharge Form	Each recruiting, continuing care, or data collection site should complete a separate form whenever that infant is discharged home, is transferred to another unit, or has died

Discontinuation of Intervention	To be completed if lactoferrin or placebo is permanently discontinued early (either by clinician or parental decision) or where parents choose to withdraw their infant from the trial
Serious Adverse Event (SAE)/SUSAR Form	Should be completed for all SAEs that are NOT 'expected' (see 9.1.6 for a list of expected events) and faxed to the NPEU CTU within 24 hours of becoming aware of the event
Incident Form	To report any deviation from the protocol, trial-specific procedures, or good clinical practice. Missed or omitted doses of lactoferrin or placebo do not need to be reported on an Incident Form as they will be captured on the Daily Dosing Log

Data collection forms will be returned to the NPEU CTU. Transfer packs will accompany infants to every hospital they are transferred to before discharge when possible or be provided by the NPEU CTU otherwise.

7.14 Withdrawal from the Trial

Parent(s) may withdraw their infant from the trial at any time; they are not obliged to give a reason. If parents choose to withdraw their infant from further administration of lactoferrin or placebo, permission will be sought to complete data collection. Parents will be asked whether data already collected may be retained and used for the purposes of the trial and their decision will be recorded on the withdrawal form. Parents will be made aware that this decision has no impact on any aspects of their infant's continuing care.

The attending clinician may withdraw the infant from treatment if they consider this to be in the best interest of the infant's health and well-being.

7.15 Inter-hospital Transfers

Participating neonatal units will be either:

1. A recruiting site where parents' consent is obtained and infants may be recruited, randomised, and commence participation in the trial.
2. A continuing care site, which will continue to administer the intervention and collect routine data if a participating infant aged less than 34 weeks' postmenstrual age is transferred in from a recruiting site.
3. A data collection site, where infants may be transferred to this site after 34 weeks' postmenstrual age and where data collection forms are completed.

From past experience, about 50% of participating infants are likely to be transferred from their recruiting neonatal care unit to a continuing care site.

7.16 Structure and Duration of Study

An initial nine month pilot study will be undertaken in selected neonatal centres within the Northern Region and Yorkshire Neonatal Networks to test whether the components and processes of the study will work together and run smoothly. Projections suggest that about 78 infants could be recruited in that time. The design of the pilot phase, if successful, will inform and be reflected in the substantive trial, and outcome data from the pilot will be included in the final analysis.

The decision to progress with the main trial will be based on efficacy, safety, and logistics and will be made in consultation with the Trial Steering Committee (TSC) and the funder. Should a decision be made not to progress to the main trial, a report on the pilot study will be submitted for publication, according to the publication policy.

The trial aims to recruit 2,200 participants from 30 neonatal care centres in the UK over a period of three years (Appendix 4).

7.17 End of Trial

The end of trial will be deemed to be when the last recruited infant has been discharged from hospital or died. An end of trial declaration will then be made to MHRA and the approving REC.

7.18 Early Cessation

Taking into consideration interim data and other evidence from relevant studies or meta-analyses, the Data Monitoring Committee (DMC) may recommend that the TSC terminate the trial if, in its view, there is proof beyond reasonable doubt that the data indicate that the trial should be terminated for all infants or for a particular subgroup of infants. Guidelines for early cessation will be agreed with the DMC and documented in the DMC Charter[47].

7.19 Follow-up

The in-hospital outcomes in this trial are considered to be of such importance to infants, families, clinicians, and services that the study is justified without needing to demonstrate longer term benefit.

If the trial does not detect statistically or clinically important differences in the in-hospital outcomes then follow-up will not be undertaken as the probability of the use of lactoferrin entering clinical practice is small. If the trial findings suggest that lactoferrin supplementation is efficacious, follow-up will be required to quantify the impact on longer term outcomes. Therefore, although assessments post-discharge from hospital are not currently planned, permission will be sought from parents to retain their personal details in a secure NPEU CTU database for follow-up to assess health and development at primary school age.

8. IMP Supply, Distribution, and Accountability

8.1 Preparation

Bulk lactoferrin will be imported from the Tatua Co-operative Dairy, Hamilton, New Zealand and individual participant packs will be assembled to GMP by the Specials Pharmacy unit of the Royal Victoria Infirmary, Newcastle-on-Tyne (manufacturer's licence number MS 17136).

8.2 Distribution

The IMP will be distributed to trial sites by the Specials Pharmacy unit of the Royal Victoria Infirmary, Newcastle-upon-Tyne.

8.3 Accountability

Drug packs will be allocated by the central randomisation system and will be recorded by NPEU CTU and the dispensing pharmacy. A record of individual administrations will be kept (See 7.13). There will be reconciliation between doses recorded as administered and doses left over after discharge.

9. Safety reporting

9.1 Definitions

9.1.1 Adverse Event (AE)

An AE or adverse event is:

Any untoward medical occurrence in a patient or clinical investigation participants administered a medicinal product, which does not necessarily have to have a causal relationship with this treatment (the study medication).

An AE can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom or disease temporally associated with the use of the study medication, whether or not considered related to the study medication.

9.1.2 Adverse Reaction (AR)

All untoward and unintended responses to a medicinal product related to any dose.

The phrase “responses to a medicinal product” means that a causal relationship between a study medication and an AE is at least a reasonable possibility, i.e. the relationship cannot be ruled out. All cases judged by either the reporting medically qualified professional or the sponsor as having a reasonable suspected causal relationship to the study medication qualify as adverse reactions.

9.1.3 Serious Adverse Event (SAE)

Adverse events are defined as serious if they:

- Result in death
- Are life-threatening
- Require inpatient hospitalisation or prolongation of existing hospitalisation
- Result in persistent or significant disability/incapacity
- Are a congenital anomaly/birth defect
- Are other important medical events

NOTE: Other events that may not result in death, are not life threatening, or do not require hospitalisation, may be considered a serious adverse event when, based upon appropriate medical judgement, the event may jeopardise the patient and may require medical or surgical intervention to prevent one of the outcomes listed above.

The term “life-threatening” 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.

9.1.4 Serious Adverse Reaction (SAR)

A serious adverse reaction is an SAE which is considered to have been caused by the administration of the trial medication. For an SAE to be considered as a reaction there must be a reasonable probability that it was related to the administration of the IMP.

9.1.5 Suspected Unexpected Serious Adverse Reaction (SUSAR)

This is a serious adverse reaction, the nature or severity of which is not consistent with the known safety profile of the trial medication (e.g. Investigator’s Brochure for an unapproved investigational product or summary of product characteristics for an approved product).

9.1.6 Expected Serious Adverse Events

The following are serious adverse events that could be reasonably expected to occur in this population of infants during the course of the trial or form part of the outcome data. They do not require reporting by the trial centres as SAEs:

- Death (unless unexpected in this population)
- Necrotising enterocolitis or focal intestinal perforation
- Bronchopulmonary dysplasia or chronic lung disease
- Intracranial abnormality (haemorrhage or focal white matter damage) on cranial ultrasound scan or other imaging
- Pulmonary haemorrhage
- Patent ductus arteriosus
- Retinopathy of prematurity

9.2 Reporting Procedures

9.2.1 Adverse Event Reporting

All expected SAEs (described above) will be recorded on the DCFs.

All other SAEs will be reported by trial sites to the NPEU CTU within 24 hours after the event has been recognised as an SAE that is not included in the list of expected SAEs. All SAE information must be recorded on an SAE reporting form and faxed to the NPEU CTU. Additional information received for a case (follow-up or corrections to the original case) needs to be detailed on a new SAE form and faxed to the NPEU CTU. A Standard Operating Procedure (SOP) outlining the reporting procedure for clinicians will be provided with the SAE form and in the trial handbook. The NPEU CTU will process and report the event as specified in its own SOPs. All SAEs will be reviewed by the DMC at regular intervals throughout the trial. The CI will inform all investigators concerned of relevant information that could adversely affect the safety of participants.

9.2.2 SUSAR Reporting

SUSARs will be reported to the Medicines and Healthcare products Regulatory Agency (MHRA) and the approving Research Ethics Committee (REC) within 7 days, if life threatening, and within 15 days for other SUSARs. In addition, a copy of the SUSAR form will be forwarded to the Chair of the DMC. The Chair will also be provided with details of all previous SUSARs with their unblinded allocation. The Chief Investigator will also inform all investigators concerned of relevant information about a SUSAR that could adversely affect the safety of participants.

9.2.3 Development Safety Update Report (DSUR)

In addition to the expedited reporting above, the CI shall submit, once a year throughout the clinical trial, or on request, a DSUR to the Competent Authority, the Ethics Committee, and the sponsor.

10. Statistics and Analysis

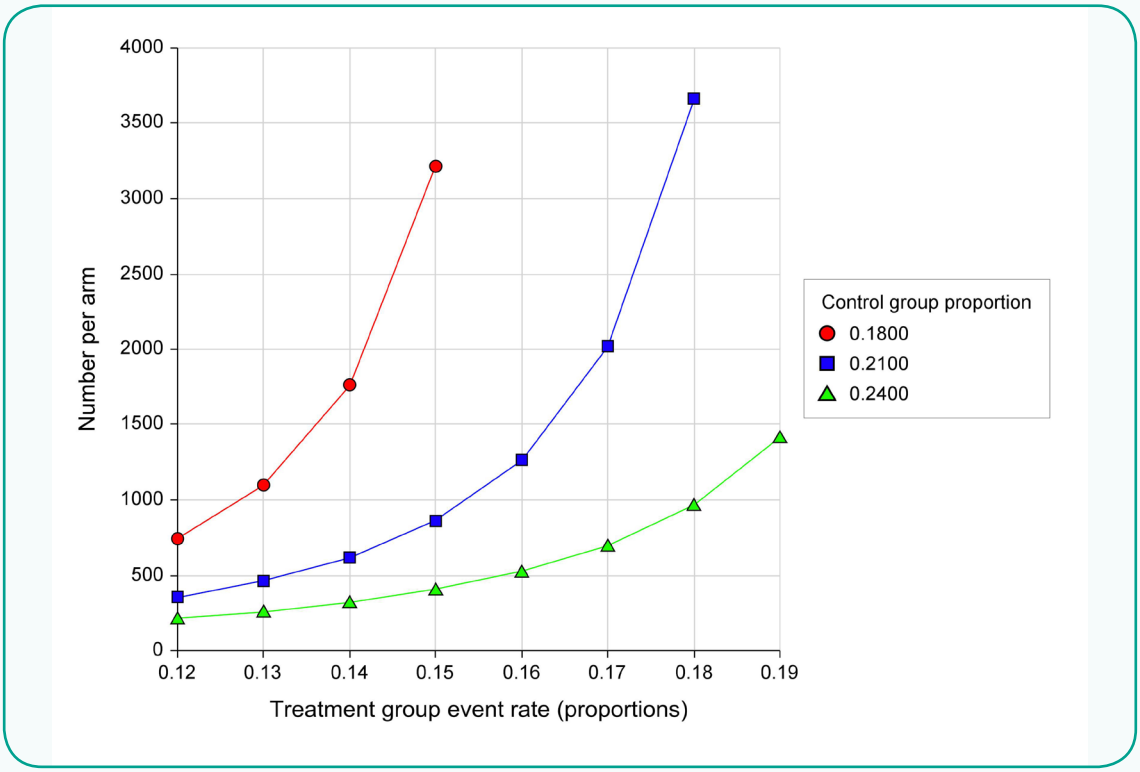
10.1 Sample Size

Calculations are based on a range of plausible primary outcome control event rates (CER) from 24% to 18%, based on estimates from Europe, North America and Australasia[2-6,36]. In summary, with 90% power and a two-sided 5% significance level, to detect an absolute risk reduction (ARR) of 5–5.8% (relative risk reduction of 24–28%) would require a trial with a total of up to 2,200 participants if the CER is 18%, 2,070 if the CER is 21%, and 2,076 if the CER is 24%. This target sample size of 2,200 will allow for an anticipated loss to follow-up of up to 5%.

The participating neonatal units admit on average 60 VLBW infants per annum. Based on 40% recruitment, 30 units will be able to recruit a total sample size of up to 2,160 infants over 3 years (an average of 2 infants per unit per month). This sample size will also be sufficient to exclude important effects on secondary outcomes with 90% power, e.g. a 7% ARR in antibiotic exposure (from 45% to 38%). The number of participants required per arm by different control event rates is shown in section 10.1.1.

10.1.1 Number of participants required per arm by different control group event rate

Control event rate	Treatment group event rate	Absolute risk reduction	Relative risk reduction	Number required per arm	Total sample size required
24%	18.2%	5.8%	24%	1,038	2,076
21%	15.5%	5.5%	26%	1,035	2,070
18%	13.0%	5.0%	28%	1,099	2,200



10.2 Statistical Analyses

Demographic factors and clinical characteristics collected as part of baseline data collection will be summarised with counts (percentages) for categorical variables, mean (standard deviation [SD]) for normally distributed continuous variables, or median (interquartile [IQR] or entire range) for other continuous variables.

Outcomes for participants will be analysed in the groups to which they are assigned regardless of deviation from the protocol or treatment received. Comparative statistical analysis will entail calculating the relative risk (RR) (95% CI) for the primary outcome (99% CIs for all other dichotomous outcomes), the mean difference (99% CI) for normally distributed continuous outcomes, or the median difference (99% CI) for skewed continuous variables.

The two groups will be compared using regression analysis, adjusting for the minimisation factors to account for the correlation between treatment groups introduced by balancing the randomisation (which forces outcomes between treatment arms to be similar apart from any treatment effect)[48]. Both the crude unadjusted and adjusted estimates will be presented, but the primary inference will be based on the adjusted analysis. Adjusted risk ratios will be estimated using a log binomial regression model, or using a log poisson regression model with a robust variance estimator if the binomial model fails to converge[49]. Linear regression will be used for normally distributed outcomes and quantile regression for skewed continuous variables.

The consistency of the effect of prophylactic enteral supplementation with lactoferrin on the primary outcome across specific subgroups of infants will be assessed using the statistical test of interaction. Pre-specified subgroup analyses include (i) week of gestation at birth, and (ii) infants given predominantly maternal or donated expressed breast milk versus formula milk (received on >50% of days in which infant is fed enterally until developing invasive infection or NEC, dying, or reaching 34 weeks' postmenstrual age).

11. Economic Analysis

The health service resources used by the infant and the family during the infant's hospital stay will be measured and evaluated. These data will be combined with clinical effectiveness data to conduct an economic evaluation assessing whether the intervention is likely to be cost-effective over the time horizon of the trial follow-up period. The analysis will take a National Health Service (NHS) perspective and will use methods consistent with those used by bodies such as the National Institute for Health and Clinical Excellence. The costs and consequences of the addition of lactoferrin will be assessed and, if appropriate, costs and consequences will be synthesised to generate an Incremental Cost-Effectiveness Ratio (ICER) to inform the adoption decision. We will use regression models to allow for differences in prognostic variables (principally gestational age bands) and other sources of heterogeneity to be accounted for and to assess whether there are differences in likely cost-effectiveness between these groups.

The primary outcome for the economic analysis will be the incidence of late-onset invasive infection. As invasive infection is linked closely to morbidity and mortality in this group, it is likely that the consequences will continue to appear over a longer time frame and may impact on both duration and quality of life. Therefore, as a second analysis, an economic model will be developed which takes into account projected longer term costs and effects on morbidity. The exact structure and specification of this model will depend on type, quality, and availability of data and cannot be pre-determined. A literature review will be conducted to inform both the structure of this model and to identify data to populate the model. The model will be used to generate estimates of the additional cost per quality-adjusted life year gained of intervention compared with placebo. The model will be subjected to extensive sensitivity analysis to assess robustness to changes in assumptions about model structure and alternative values of key parameters.

12. Direct Access to Source Data/Documents

Direct access to source data/documents (including hospital records, clinical charts, laboratory notes, memoranda, pharmacy dispensing records, recorded data from automated instruments, test reports, subject files, and records kept at the pharmacy, at the laboratories and at hospital departments involved in the clinical trial) will be granted to authorised representatives from the study organisers, the research sponsor,NHS Trusts/NHS Boards/Health and Social Care Trusts and regulatory authorities to permit trial-related monitoring, audits, and inspections.

13. Quality Control and Assurance

13.1 Risk Assessment

NPEU CTU has performed a risk assessment of the trial prior to commencement that will be reviewed at regular intervals according to its own SOP. Based on the assessment of the available toxicology and the fact that as a milk derived foodstuff, lactoferrin has been part of the human diet for thousands of years, it is proposed that the trial be considered to be of Type B (risk somewhat above that of normal clinical practice).

13.2 National Registration Systems

All surviving infants recruited into ELFIN will be 'flagged' after discharge to confirm status using records held and maintained by the The Health and Social Care Information Centre and other central UK NHS bodies.

13.3 Site Initiation and Training

Start-up visits at each participating neonatal care centre to ensure training in trial procedures will be performed before recruitment of infants is permitted. Regular site visits will be made by the Local Research Nurse (LRN) to ensure adherence to the protocol and to deal with any specific site issues.

Nurse study days will be undertaken to ensure that nurses involved with the study are fully apprised of issues such as informed consent, data collection, follow-up, and changing regulations. Meetings for Principal Investigators and nurses will be organised when workshops to discuss protocol issues, data collection issues, and study specific procedures are conducted.

13.4 Data Collection and Processing

All trial data will be collected using bespoke DCFs. Data will be processed in line with the NPEU CTU Data Management SOPs, using validated data management systems to ensure consistency, viability, and quality of the data. It is then stored in line with the Data Protection Act (1998).

13.5 Central Statistical Monitoring

Central statistical monitoring will be used to monitor patterns of recruitment at sites and within the data; outlier data will be investigated and may trigger 'for cause' site monitoring.

The NPEU CTU Director and the Senior Trials Programmer will develop an appropriate central monitoring plan for the trial and review the output to identify any unexpected patterns or problems. The Head of Trials will decide if any action needs to be taken.

13.6 Site Monitoring and Auditing

Each recruiting centre will be staffed by 0.2 WTE Local Research Nurse (LRN). The LRN will be responsible for the day-to-day smooth running of the trial at a recruiting site. They will encourage recruitment, provide staff education and training, and monitor data collection completeness and quality.

The LRN will submit formal site visit reports to the Project Management Group (PMG). No other routine monitoring will be carried out unless the central monitoring exercises raise cause for concern. Likewise, sites will only be audited if central monitoring indicates a necessity. This monitoring approach is justified by the level of risk associated with the trial and the IMP.

14. Serious Breaches

The Medicines for Human Use (Clinical Trials) Regulations contain a requirement for the notification of "serious breaches" to the MHRA within 7 days of the sponsor becoming aware of the breach.

A serious breach is defined as "A breach of GCP or the trial protocol which is likely to effect to a significant degree –

a) the safety or physical or mental integrity of the subjects of the trial; or

b) the scientific value of the trial".

In the event that a serious breach is suspected the NPEU CTU centre should be contacted as soon as possible. NPEU CTU will refer serious breaches to the sponsor immediately.

15. Ethics

15.1 Declaration of Helsinki

The Investigator will ensure that this study is conducted in accordance with the principles of the Declaration of Helsinki.

15.2 ICH Guidelines for Good Clinical Practice

The Investigator will ensure that this study is conducted in full conformity with relevant regulations and with the ICH Guidelines for Good Clinical Practice (CPMP/ICH/135/95) July 1996.

15.3 Approvals

The trial will only start after gaining approval from the MHRA and a National Research Ethics Service (NRES) registered ethics committee. Additionally, approval of the Trust R&D Office will be sought for individual trial sites.

Applications will be submitted through the Integrated Research Application System (IRAS).

A copy of the protocol, parent information leaflet, GP letter, and informed consent form will be submitted to the MHRA and the REC for approval.

The CI or their delegate will submit and, where necessary, obtain approval from the REC for any protocol amendments and changes to the informed consent document.

The CI or their delegate will notify any protocol deviations to the sponsor and will notify the REC of these in accordance with local procedures.

15.4 Participant Confidentiality, Data Handling and Record Keeping

SOPs are in place for the collection and handling of data received at the NPEU CTU. The CI will take overall responsibility for ensuring that each participant's information is kept confidential. All paper documents will be stored securely and kept in strict confidence in compliance with the Data Protection Act (1998). Data collected on the DCFs will be transferred for storage in an electronic database in which the participant will be identified only by a trial specific number. The infant's name and any other identifying details will be stored in a separate database linked only by the trial number. This information will be collected and retained with the parent's explicit consent to enable follow-up to be undertaken. After the trial has been completed and the reports published, the data will be archived in a secure physical or electronic location with controlled access.

Storage will be on a restricted area of a file server. The server is in a secure location and access is restricted to a few named individuals. Access to the building in which the NPEU CTU is situated is via an electronic tag and individual rooms are kept locked when unoccupied. Authorisation to access restricted areas of the NPEU network is as described in the NPEU security policy.

Data will be processed on a workstation by authorised staff. The computer workstations access the network via a login name and password (changed regularly). No data are stored on individual workstations. Backing up is done automatically overnight to an offsite storage area. The location of the back-up computer is in a separate department which has electronic tag access. Access to the room in which the back-up machine is located is via a key-pad system.

15.5 Retention of Personal Data

Personal data will be needed to re-contact parents when their children are around 5 years of age, to co-ordinate follow up, and to disseminate the results of the follow up study to parents.

16. Funding

The National Institute for Health Research (NIHR) Health Technology Assessment (HTA) programme is funding the trial.

17. Insurance

Indemnity will be provided against both negligent and non-negligent harm as defined below:

- Negligent Harm: The University has arrangements in place to provide for harm arising from participation in the study for which the University is the Research Sponsor. NHS indemnity operates in respect of the clinical treatment which is provided.
- Non-Negligent Harm: The University has arrangements in place to provide for non-negligent harm arising from participation in the study for which the University is the Research Sponsor.

18. Trial Governance

18.1 Site Research and Development Approval

Individual sites will only commence recruiting participants once they receive approval from NHS Trust Research and Development (R&D) Offices. Applications to R&D offices will be submitted through the NIHR Co-ordinated System for gaining NHS permission.

18.2 Trial Sponsor

The University of Oxford is the nominated sponsor for the trial.

18.3 Co-ordinating Centre

The trial co-ordinating centre will be at the NPEU CTU, University of Oxford where the Trial Co-ordinator will be based. The NPEU CTU will be responsible for all trial programming, randomisation, data entry and management, conducting statistical analyses, servicing both the DMC and TSC, and, in collaboration with the CI and the Trial Research Nurse, for the day-to-day running of the trial including recruitment of centres and training of staff.

18.4 Project Management Group

The trial will be supervised on a day-to-day basis by the PMG. This group reports to the TSC which is responsible to the trial sponsor. At each participating centre, a local PI will report to the PMG via the project funded staff based at the NPEU CTU.

The core PMG will consist of the CI and NPEU CTU staff including:

- NPEU CTU Director
- Senior Trials Manager
- Senior Trials Programmer
- Trial Coordinator
- Trial Statistician
- Trial Programmer
- Administrator/Data Manager

The core PMG will meet regularly (at least monthly). Every 3–4 months the Clinical Investigators' Group, (CIG) will meet. This will comprise all co-applicants and the members of the core PMG.

18.5 Trial Steering Committee

The trial will be overseen by a TSC consisting of an independent chair and at least two other independent members.

Representatives from relevant Patient / Public Involvement groups and the CI will be joined by observers from the NPEU CTU. The HTA programme manager will be invited to attend all TSC meetings.

18.6 Data Monitoring Committee

A DMC independent of the applicants and of the TSC will review the progress of the trial at least annually and provide advice on the conduct of the trial to the TSC and (via the TSC) to the HTA programme manager. The DMC will act according to its Charter, which will be agreed at its first meeting.

18.7 Conflicts of Interest

In 2011 Dr Embleton provided advice to Baxter, a company who make parenteral nutrition solutions for neonates. The honoraria received was donated to charity. He has no ongoing relationships with this or any other relevant commercial organisation and does not disclose any other relevant conflicts of interest.

The ELFIN co-applicants confirm that they have no other competing interests or affiliations to declare. Principal investigators at recruiting sites will be asked to state any potential conflicts of interest, and these will be declared when the results of the trial are published.

19. Communication

19.1 Protocol

After REC and MHRA approval has been obtained, this protocol will be submitted for publication and will be available for download via the NPEU website.

19.2 Post-recruitment Information for Parents and 'On-going consent'

Parents will be offered an early appointment with the PI or delegated deputy to ensure they understand the trial procedures and continue to consent to participate in the trial.

19.3 Post-discharge Information

Information about the study will continue to be offered to parents after their infant leaves the neonatal unit. A regular newsletter will be produced giving parents information about the study until it has finished. Experience with other studies in this area suggests that parents of infants who die may want to receive these newsletters, and all parents will be offered the chance to receive correspondence or opt out.

19.4 Study Findings

The CI will co-ordinate dissemination of the results from this study. All publications using data from this study to undertake original analyses will be submitted to the TSC for review before release. To safeguard the scientific integrity of the trial, data from this study will not be presented in public before the main results are published without the prior consent of the TSC.

19.5 Publication Policy/Acknowledgement of Contribution

The success of the trial depends on a large number of neonatal nurses, neonatologists, and parents. Credit for the study findings will be given to all who have collaborated and participated in the study including all local co-ordinators and collaborators, members of the trial committees, the NPEU CTU, and trial staff. Authorship at the head of the primary results paper will take the form "The Enteral Lactoferrin in Neonates (ELFIN) Collaborative Group" to avoid giving undue prominence to any individual. The writing will be the responsibility of a writing committee including all of the investigators. All contributors to the study will be listed at the end of the report, with their contribution to the study identified.

Those responsible for other publications reporting specific aspects of the study, such as detailed microbiological outcomes, may wish to utilise a different authorship model, such as "[name], [name] and [name] on behalf of the "The ELFIN Collaborative Group". Decisions about authorship of additional papers will be discussed and agreed by the trial investigators and the TSC.

Parents will be sent a summary of trial publications if they wish, which will contain full references. A copy of the journal article will be available on request from the CI.

20. References

1. Fanaroff AA, Stoll BJ, Wright LL, et al. Trends in neonatal morbidity and mortality for very low birthweight infants. Am J Obstet Gynecol 2007; 196:147.e1-8.
2. Stoll BJ, Hansen N. Infections in VLBW infants: studies from the NICHD Neonatal Research Network. Sem Perinatol 2003;27:293-301.
3. Vergnano S, Menson E, Kennea N, et al. Neonatal infections in England: the NeonIN surveillance network. Arch Dis Child Fetal Neonatal Ed 2011;96:F9-F14.
4. Isaacs D; Australasian Study Group for Neonatal Infections. A ten year, multi-centre study of coagulase negative staphylococcal infections in Australasian neonatal units. Arch Dis Child Fetal Neonatal Ed 2003;88:F89-93.
5. Gordon A, Isaacs D. Late onset neonatal Gram-negative bacillary infection in Australia and New Zealand: 1992-2002. Pediatr Infect Dis J 2006;25:25-9.
6. Isaacs D, Fraser S, Hogg G, Li HY. Staphylococcus aureus infections in Australasian neonatal nurseries. Arch Dis Child Fetal Neonatal Ed 2004:F331-5.
7. Clerihew L, Lamagni TL, Brocklehurst P, McGuire W. Invasive fungal infection in very low birthweight infants: national surveillance study. Arch Dis Child Fetal Neonatal Ed 2006;91:188-92.
8. Chapman RL, Faix RG. Persistent bacteremia and outcome in late onset infection among infants in a neonatal intensive care unit. Pediatr Infect Dis J 2003;22:17-21.
9. Adams-Chapman I, Stoll BJ. Neonatal infection and long-term neurodevelopmental outcome in the preterm infant. Curr Opin Infect Dis 2006;19:290-7.
10. Bassler D, Stoll BJ, Schmidt B, et al. Using a count of neonatal morbidities to predict poor outcome in extremely low birth weight infants: added role of neonatal infection. Pediatrics 2009;123:313-8.
11. Stoll BJ, Hansen NI, Adams-Chapman I, et al. Neurodevelopmental and growth impairment among extremely low-birth-weight infants with neonatal infection. JAMA 2004;292:2357-65.
12. Fowlie PW, Schmidt B. Diagnostic tests for bacterial infection from birth to 90 days-a systematic review. Arch Dis Child Fetal Neonatal Ed 1998;78:F92-8.
13. Malik A, Hui CP, Pennie RA, Kirpalani H. Beyond the complete blood cell count and C-reactive protein: a systematic review of modern diagnostic tests for neonatal sepsis. Arch Pediatr Adolesc Med 2003;157:511-6.
14. Buttery JP. Blood cultures in newborns and children: optimising an everyday test. Arch Dis Child Fetal Neonatal Ed 2002;87:F25-8.
15. Gordon A, Isaacs D. Late-onset infection and the role of antibiotic prescribing policies. Curr Opin Infect Dis 2004;17:231-6.
16. Isaacs D. Unnatural selection: reducing antibiotic resistance in neonatal units. Arch Dis Child Fetal Neonatal Ed 2009;91:F72-4.
17. Murphy BP, Armstrong K, Ryan CA, Jenkins JG. Benchmarking care for very low birthweight infants in Ireland and Northern Ireland. Arch Dis Child Fetal Neonatal Ed 2010;95:F30-5.
18. Lee SK, Aziz K, Singhal N, et al. Improving the quality of care for infants: a cluster randomised controlled trial. CMAJ 2009;181:469-76.
19. Scott PH. Plasma lactoferrin levels in newborn preterm infants: effect of infection. Ann Clin Biochem 1989;26:412-5.
20. Embleton NE, Pang N, Cooke RJ. Postnatal malnutrition and growth retardation: an inevitable consequence of current recommendations in preterm infants? Pediatrics 2001;107:270-3.
21. Wiesner J, Vilcinskas A. Antimicrobial peptides: the ancient arm of the human immune system. Virulence 2010;1:440-64

22. Ochoa TJ, Cleary TG. Effect of lactoferrin on enteric pathogens. Biochimie 2009;91:30-4.
23. Valenti P, Antonini G. Lactoferrin: an important host defence against microbial and viral attack. Cell Mol Life Sci 2005;62:2576-87.
24. Legrand D, Pierce A, Elass E, et al. Lactoferrin structure and functions. Adv Exp Med Biol 2008;606:163- 94.
25. Gonzalez-Chavez SA, Arévalo-Gallegos S, Rascón-Cruz Q. Lactoferrin: structure, function and applications. Int J Antimicrob Agents 2009;33:301.e1-8.
26. Tian H, Maddox IS, Ferguson LR, Shu Q. Influence of bovine lactoferrin on selected probiotic bacteria and intestinal pathogens. Biometals 2010;23:593-6.
27. Actor JK, Hwang SA, Kruzel ML. Lactoferrin as an immune modulator. Curr Pharm Des 2009;15:1956-73.
28. Berlutti F, Schippa S, Morea C, et al. Lactoferrin downregulates pro-inflammatory cytokines upexpressed in intestinal epithelial cells infected with invasive or noninvasive E. coli strains. Biochem Cell Biol 2006;84:351-7.
29. Wakabayashi H, Takakura N, Yamauchi K, Tamura Y. Modulation of immunity-related gene expression in small intestines of mice by oral lactoferrin. Clin Vaccine Immunol. 2006; 13: 239-45.
30. Buccigrossi V, de Marco G, Bruzzese E, et al. Lactoferrin induces concentration-dependent functional modulation of intestinal proliferation and differentiation. Pediatr Res. 2007; 61: 410-4.
31. Raghuveer TS, McGuire EM, Martin SM, et al. Lactoferrin in the preterm infants' diet attenuates iron-induced oxidation products. Pediatr Res.2002;52:964-72.
32. Magnuson JS, Henry JF, Yip TT, Hutchens TW. Structural homology of human, bovine, and porcine milk lactoferrins: evidence for shared antigenic determinants. Pediatr Res 1990;28:176-81.
33. Vorland LH, Ulvatne H, Andersen J, et al. Lactoferricin of bovine origin is more active than lactoferricins of human, murine and caprine origin. Scand J Infect Dis 1998;30:513-7.
34. Office of Food Additive Safety. GRAS notification. Food and Drug Administration. http://www.accessdata.fda.gov/scripts/fcn/gras_notices/204568A.pdf28
35. Lönnerdal B. Nutritional and physiologic significance of human milk proteins. Am J Clin Nutr. 2003; 77:1537S-43S.
36. Manzoni P, Rinaldi M, Cattani S, et al. Bovine lactoferrin supplementation for prevention of late-onset sepsis in very low-birth-weight neonates: a randomized trial. JAMA 2009;302:1421-8.
37. Venkatesh MP, Abrams SA. Oral lactoferrin for the prevention of sepsis and necrotizing enterocolitis in preterm infants. Cochrane Database Syst Rev 2010;5:CD007137.
38. Kaufman DA. Lactoferrin supplementation to prevent nosocomial infections in preterm infants. JAMA 2009;302:1467-8
39. Askie LM, Brocklehurst P, Darlow BA, et al, the NeOProM Collaborative Group: NeOProM: neonatal oxygenation prospective meta-analysis collaboration study protocol. BMC Pediatr 2011;11:6.
40. European Medicines Agency. Report on the Expert Meeting on Neonatal and Paediatric Sepsis 2010. http://www.ema.europa.eu/docs/en_GB/document_library/Report/2010/12/WC500100199.pdf
41. Modi N, Doré CJ, Saraswatula A, et al. A case definition for national and international neonatal bloodstream infection surveillance. Arch Dis Child Fetal Neonatal Ed 2009;94:F8-12.
42. Walsh MC, Kliegman RM. Necrotizing enterocolitis: treatment based on staging criteria. Pediatr Clin North Am 1986;33:179-201.
43. ICROP. An International Classification of Retinopathy of Prematurity. Pediatrics 1984;74:127-33.

44. Ehrenkranz RA, Walsh MC, Vohr BR, et al. Validation of the National Institutes of Health consensus definition of bronchopulmonary dysplasia. Pediatrics 2005;116:1353-60.
45. British Association of Perinatal Medicine. Categories of care 2011 (August 2011). http://www.bapm.org/publications/documents/guidelines/CatsofcarereportAug11.pdf
46. Allmark P, Spedding M. Clinical trials in neonates: ethical issues. Semin Fetal Neonatal Med 2007;12:318-23.
47. DAMOCLES Study Group, NHS Health Technology Assessment Programme. A proposed charter for clinical trial data monitoring committees: helping them to do their job well. Lancet 2005;365:711-22.
48. Kahan BC, Morris TP. Analysis of multi-centre trials with continuous outcomes: when and how should we account for center effects? Statist. Med. 2012; DOI: 10.1002/sim.5667
49. Yelland LN, Salter AB, Ryan P. Relative risk estimation in cluster randomized trials: A comparison of generalized estimating equation methods. 2011;7:1-26.

21. Appendix 1: Case Definition of Necrotising Enterocolitis

NEC may be diagnosed at surgery, at post-mortem examination or clinically and radiologically using the following criteria:

At least one of the following clinical signs present:

- **Bilious** gastric aspirate or emesis
- Abdominal distension
- Occult or gross **blood in stool (no fissure)**

and at least one of the following radiological features:

- Pneumatosis intestinalis
- Hepato-biliary gas
- Pneumoperitoneum

Infants who satisfy the definition of NEC above but are found at surgery or post-mortem examination for that episode to have a “Focal Gastrointestinal Perforation” should be coded as having “Focal Gastrointestinal Perforation”, not as having NEC.

22. Appendix 2: Classification of Categories of Care

INTENSIVE CARE

General principle: This is care provided for infants who are the most unwell or unstable and have the greatest needs in relation to staff skills and staff to patient ratios.

Definition of Intensive Care Day

- Any day where an infant receives any form of mechanical respiratory support via a tracheal tube
- **BOTH** non-invasive ventilation (e.g. nasal CPAP) and parenteral nutrition
- • Day of surgery (including laser therapy for ROP)
- • Day of death
- • Any day receiving any of the following
    - Presence of an umbilical arterial line
    - Presence of an umbilical venous line
    - Presence of a peripheral arterial line
    - Insulin infusion
    - Presence of a chest drain
    - Exchange transfusion
    - Therapeutic hypothermia
    - Prostaglandin infusion
    - Presence of replogle tube
    - Presence of epidural catheter
    - Presence of silo for gastroschisis
    - Presence of external ventricular drain
    - Dialysis (any type)

HIGH DEPENDENCY CARE

General principle: This is care provided for infants who require highly skilled staff but where the ratio of nurses to patients is less than intensive care.

Definition of High Dependency Care Day

- Any day where an infant does not fulfil the criteria for intensive care where any of the following apply:
- Any day where an infant receives any form of non invasive respiratory support (e.g. nasal CPAP)
- Any day receiving any of the following:
    - parenteral nutrition
    - continuous infusion of drugs (except prostaglandin &/or insulin)
    - presence of a central venous or long line (PICC)
    - presence of a tracheostomy
    - presence of a urethral or suprapubic catheter
    - presence of trans-anastomotic tube following oesophageal atresia repair
    - presence of NP airway/nasal stent
    - observation of seizures or cerebral function monitoring
    - barrier nursing
    - ventricular tap

SPECIAL CARE

General principle: Special care is provided for infants who require additional care delivered by the neonatal service but do not require either Intensive or High Dependency care.

Definition of Special Care Day

- Any day where an infant does not fulfil the criteria for intensive or high dependency care and requires any of the following:
  - oxygen by nasal cannula
  - feeding by nasogastric, jejunal tube or gastrostomy
  - continuous physiological monitoring (excluding apnoea monitors only)
  - care of a stoma
  - presence of IV cannula
  - infant receiving phototherapy
  - special observation of physiological variables at least 4 hourly

23. Appendix 3: IMP Management

Trial medication:

- **Lactoferrin** will be packaged into 25 ml opaque pharmacy pots (fill equivalent to 375 mg active lactoferrin per pot) at the trial pharmacy in Newcastle Royal Infirmary. Boxes containing 24 identically numbered pots will be labelled with the same pack ID number to indicate that they all belong to the same treatment course. At randomisation, infants will be allocated a study number and a pack ID number; the study number will be added to the label of the allocated pack along with the infant's name and date of birth which is checked before each administration of study product. Lactoferrin powder is stable within unopened pots and may be stored at room temperature. When the infant has completed the course of treatment, either at 34 completed weeks or at death or discharge from hospital, any unused product should be retained for collection by the study team.
- The dose will be prepared and administered by neonatal nurses or clinicians on the neonatal unit (in the milk kitchen or other appropriate area determined locally).
- **Sucrose placebo** will be processed, packaged and distributed as for lactoferrin.

Preparation of product for administration:

1. Verify that the Pack ID number on the pharmacy pot matches the Pack ID allocated to the infant (it will be stated on the randomisation confirmation email and should be recorded on the Daily Dosing Log).
2. Add 4 ml of sterile water (supplied in plastic vial) plus 1 ml of either expressed breast milk (EBM) or formula (if EBM is not available) to the pharmacy pot which contains 375 mg of either lactoferrin or sucrose placebo.
3. Seal the pot with the lid and shake vigorously by hand for 30 seconds.
4. Leave the pot at room temperature for 30 minutes.
5. Using a syringe, draw off suspension (2 ml/kg body weight up to a maximum of 4 ml) for naso/oro-gastric or oral administration (via spoon/cup/syringe or bottle). Participating centres will be supplied with oral syringes if their standard oral syringe is not compatible with the lactoferrin/placebo pot insert.
6. Study medication is normally given once daily. For very small infants, clinicians or caregivers may choose to administer the daily dose in two aliquots. If these are to be given more than 30 minutes apart, then a fresh dose should be prepared as above for each.
7. If there is any concern about acute intestinal inflammation or perforation then the dose will be omitted. Whether doses are omitted at other times when the infant is unwell or demonstrates enteral feeds intolerance will be at the discretion of the attending consultant paediatrician.

24. Appendix 4: Recruitment Projections

Stage	Dates (incl)	Number of recruiting sites	Recruits
Pilot study	Dec 2013 - Aug 2014	6	82
Main trial (Year 1)	Sept 2014 - Aug 2015	30	600
Main trial (Year 2)	Sept 2015 - Aug 2016	30	1400
Main trial (Year 2)	Sept 2016 - Aug 2017	30	2200

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