CLINICAL STUDY PROTOCOL

(IMPERIAL CLINICAL TRIALS UNIT (ICTU) ADOPTED)

Full study title: An efficient, UK-wide, real-world-data-enabled, adaptive, 2-randomisation, controlled trial to determine clinical efficacy, effect size, and safety of widely used enteral feeds in reducing necrotising enterocolitis, mortality, and cognitive impairment in preterm babies born below 29 weeks' gestation

Short study title: COLLABORATE

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This protocol has regard for HRA guidance

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This protocol describes the COLLABORATE study and provides information about procedures for enrolling participants. Every care was taken in its drafting, but corrections or amendments may be necessary. These will be circulated to investigators in the study, but centres enrolling participants for the first time are advised to contact the trial coordination centre to confirm they have the most recent version. Problems relating to this study should be referred, in the first instance, to the trial coordination centre.

This trial will adhere to the principles outlined in the International Conference on Harmonisation Good Clinical Practice guidelines. It will be conducted in compliance with the protocol, the Data Protection Act 2018 and other regulatory requirements as appropriate.

ABBREVIATIONS

AE	Adverse Event
AR	Adverse Reaction
CI	Chief Investigator
DMEC	Data Monitoring and Ethics Committee
eCRF	Electronic Case Report Form
HRA	Health Research Authority
ICMJE	International Committee of Medical Journal Editors
ICTU	Imperial Clinical Trials Unit
MCID	Minimum Clinically Important Difference
MRI	Magnetic Resonance Imaging
NEC	Necrotising Enterocolitis
NHS	National Health Service
NICE	National Institute for Health and Care Excellence
NNRD	National Neonatal Research Database
PARCA-R	Parent Report of Children's Abilities-Revised
pHDM	Pasteurised Human Donor Milk
PMA	Post menstrual age
PPPIE	Parent-Patient-Public Engagement and Involvement
QA	Quality Assurance
REC	Research Ethics Committee
RCT	Randomised Controlled Trial
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SAR	Serious Adverse Reaction
TMG	Trial Management Group
TSC	Trial Steering Committee

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TRIAL SUMMARY

TITLE

An efficient, UK-wide, real-world-data-enabled, adaptive, 2-randomisation, controlled trial to determine clinical efficacy, effect size, and safety of widely used enteral feeds in reducing necrotising enterocolitis, mortality, and cognitive impairment in preterm babies born below 29 weeks' gestation

OBJECTIVES

- To assess, in babies born <29 weeks gestation, the efficacy of pasteurised Human Donor Milk (pHDM) compared with Preterm Formula, when used as a supplement should there be insufficient milk from their own mother (Own Mother's Milk), on "survival to 34 weeks postmenstrual age without surgical necrotising enterocolitis (NEC)" (primary outcome), language and cognitive development at age 2-years, and other outcomes
- To assess, in babies born <29 weeks gestation, the efficacy of routine cow-milk based protein-carbohydrate fortification of human milk feeds (Own Mother's Milk and pHDM) compared with no routine fortification on "survival to 34 weeks postmenstrual age without surgical necrotising enterocolitis (NEC)" (primary outcome), language and cognitive development at age 2-years, and other outcomes
- To determine if pHDM and Preterm Formula exert different effects on neurodevelopment through the mechanism of altered cerebral white matter microstructure
- To establish if the additional cost of pHDM to the NHS is justified through a reduction in NEC

DESIGN

An adaptive, UK-wide, real-world-data-enabled, digital-technology facilitated, 2-randomisation, open-label, controlled trial. Infants may participate in either or both randomisations.

SAMPLE SIZE

This is an adaptive trial; hence the sample size is not fixed. The maximum sample size is 3252, assuming no early stopping and based on the following assumptions: i) Minimum Clinically Important Difference (MCID) of 0.05 (from 0.87 to 0.82) in the proportion "survival to 34 weeks gestation without surgical NEC" (primary outcome); ii) 2- sided alpha of 0.05; iii) 87% power with three planned looks at the data with stopping rules for efficacy (at 50%, 75%, and 100% recruitment); iv) maximum 2% withdrawal rate of consent to use National Neonatal Research Database (NNRD) data; v) 50% co-enrolment rate.

Mechanistic study: 120 infants provide 80% power (2-sided; alpha=0.05) to detect a 10% difference in fractional anisotropy using 3T brain MRI tract-based spatial statistics (measure of white matter microstructure).

INCLUSION/EXCLUSION CRITERIA

Randomisation 1

Inclusion

- Born <29 weeks gestation

- No condition precluding enteral feeding
- Maternal intention to express breast milk
- Parent/guardian verbal consent

Exclusion

- Baby has already received pHDM or Preterm Formula

Randomisation 2

Inclusion

- Born <29 weeks gestation
- No condition precluding enteral feeding
- Maternal intention to express breast milk
- Parent/guardian verbal consent

Exclusion

- Baby has already received Fortifier

Mechanistic studyInclusion

- Participants in randomisation 1 recruited at the Royal Infirmary of Edinburgh
- Parent/guardian written consent

Exclusion

- Infants with congenital anomalies: structural or functional (e.g., metabolic disorders) that occur during intrauterine life and can be identified prenatally, at birth or later in life (WHO definition)
- Infants in whom MRI at 3Tesla is contraindicated

INTERVENTIONS and COMPARATORS

Randomisation1

Intervention

- pHDM from a UK human milk bank

Comparator

- Standard commercial Preterm Formula

Randomisation 2

Intervention

- Routine addition of commercial protein-carbohydrate cow-milk derived fortifier to human milk feeds

Comparator

- No routine use of fortifier

OUTCOME MEASURES

Primary

Assessed at 34 weeks postmenstrual age

- Survival without surgical NEC

Secondary

Assessed at 34 weeks postmenstrual age

- Survival
- Surgical NEC
- Spontaneous Intestinal Perforation

Assessed at 36 weeks postmenstrual age

- Bronchopulmonary dysplasia

Assessed at postnatal age 28 days

- Survival

Assessed at neonatal unit discharge (or death)

- Survival
- Surgery for NEC or NEC related condition after 34 weeks postmenstrual age
- Medical NEC
- Age in days to achieve an enteral intake of 150 ml/kg/day
- Treated retinopathy of prematurity
- Severe brain injury
- Any diagnosis of milk-curd obstruction
- Length of neonatal unit stay
- Number of episodes of bacterial or fungal bloodstream infection
- Number of episodes of bacterial or fungal cerebrospinal fluid infection
- Number of episodes of bacterial or fungal urinary tract infection
- Number of days of antibiotic treatment
- Number of days on parenteral nutrition
- Number of days nil by mouth
- Weight, length, and head circumference Z-scores
- Change from birth in Z-scores for weight, length, and head circumference
- Any breast feeding (suckling at breast)
- Exclusive breast feeding (suckling at breast)
- Receiving any expressed Own Mother's Milk
- Receiving exclusive expressed Own Mother's Milk
- Maximum serum urea, creatine, and alkaline phosphatase
- Health resource use

Assessed at neonatal unit discharge (or death) in babies with NEC surgery

- Drain insertion prior to surgery (yes/no)
- Diagnosis of short bowel syndrome (yes/no)
- Diagnosis of intestinal failure-associated liver disease (yes/no)
- Length of bowel resected (cm)

- Primary surgical procedure (ileostomy; colostomy; end-to-end anastomosis)
- Number of re-operations (excluding primary operation)

Assessed at age 2-years corrected for prematurity

- Survival without moderate-severe cognitive-language impairment
- Survival
- Moderate-severe cognitive-language impairment
- Cognitive sub-score
- Language sub-score
- Gross motor function
- Hearing impairment
- Vision impairment

1 BACKGROUND

1.1 Clinical setting

The study will take place in NHS neonatal units in the UK. The eligible population comprises babies born less than 29 weeks gestation.

1.2 Intervention and comparator details

The intervention for randomisation 1 is pasteurised Human Donor Milk (pHDM), obtained from one of 15 human milk banks in the country that operate on a charitable or not-for-profit basis. The intervention for randomisation 2 is multicomponent fortifier, a commercial product available as a powder added to human milk (Own Mother's Milk and pHDM), that is prepared from cow milk and contains protein, carbohydrate, minerals, vitamins, and trace elements. Fortifiers used in the UK are standard hospital stock. A few UK neonatal units are using commercial fortifier made from pooled human milk.

The comparator for randomisation 1 is preterm formula (standard hospital stock). These are highly processed products constituted from cow-milk to provide a higher nutrient density than human milk. They also contain vegetable fats, vitamins, and minerals. The composition of preterm formula is strictly regulated following guidelines set by the European Union and Codex Alimentarius Commission, a joint World Health Organisation/Food and Agriculture Organisation of the United Nations, established to protect consumer health and promote fair food trade practices. The comparator for randomisation 2 is no routine fortification.

1.3 Hypotheses

P: population, I: intervention, C: comparator, PO primary outcome

1.3.1 Randomised trial hypotheses

- In babies born <29 weeks gestation for whom there is need to supplement Own Mother's Milk because the volume available is insufficient (P), pHDM (I), when compared with Preterm Formula (C), improves survival rate to 34 weeks postmenstrual age without surgical NEC (PO).
- In babies born <29 weeks gestation (P), routine macronutrient fortification of human milk (Own Mother's Milk and pHDM) with cow milk fortifier (I), when compared with no routine fortification (C), improves survival rate to 34 weeks postmenstrual age without surgical NEC (PO).

1.3.2 Mechanistic hypotheses

- In babies born <29 weeks gestation for whom there is a need to supplement Own Mother's Milk because the volume available is insufficient (P), pHDM (I), when compared with Preterm Formula (C), differs in effect on the maturation of cerebral white matter tracts that subserve cognitive function (PO), assessed at full-term.
- In babies born <29 weeks gestation for whom there is a need to supplement Own Mother's Milk because the volume available is insufficient (P), the use of pHDM (I) compared with Preterm Formula (C) results in a different gut microbiota composition and/or diversity (PO).

1.3.3 NHS value hypothesis

- In babies born <29 weeks gestation for whom there is a need to supplement Own Mother's Milk because the volume available is insufficient (P), the additional cost of pHDM (I), when

compared with Preterm Formula (C), generates savings through improved survival to 34 weeks postmenstrual age without surgical NEC (PO).

- In babies born <29 weeks gestation (P) who receive routine (I) versus no routine (C) fortification of human milk feeds, the additional cost of fortifier, generates savings through improved survival to age two-years corrected for preterm birth without moderate-severe cognitive-language impairment (PO).

1.4 Rationale for the study

The rationale for this study is that the types of enteral feeds an extremely preterm baby receives, namely fresh Own Mother's Milk, pHDM, Preterm Formula, and macronutrient fortifier, are widely believed to influence the risk of necrotising enterocolitis (NEC), and because nutrition, and the non-nutritional components of human milk are highly likely to affect the development of the preterm brain. However, pHDM, Preterm Formula, and macronutrient fortifier have entered into widespread use without evidence of efficacy or safety.

NEC is an acquired inflammation of the immature gastrointestinal tract and leading cause of death and life-long impairment in extremely preterm babies in high-income settings such as the UK. There are around 3000 babies born <29 weeks gestation, and around 260 NEC deaths and/or surgeries each year in the UK, with up to 3 times more developing less severe disease that nonetheless disrupts feeding, prolongs hospital stay, and increases antibiotic exposure, and costs of care (1).

Own Mother's Milk is standard of care for all newborn babies and reduces but does not totally prevent NEC (1). Own Mother's Milk and pHDM are not equivalent as not only is human milk very variable in nutrient and non-nutrient content, but composition is unique to each mother, and pasteurisation substantially reduces or destroys biologically active non-nutritional components (2).

The developmental ability to suckle effectively is not reached until 33-34 weeks postmenstrual age, hence mothers delivering preterm must express breast milk for many weeks which they report as stressful and difficult to sustain. Therefore, over 85% of extremely preterm babies require supplementary feeds because a mother is unable to express sufficient milk to meet her baby's needs. Often, the shortfall is only temporary. Overall, in the UK, around a third of extremely preterm infants requiring supplementary feeds receive pHDM and around two-thirds receive Preterm Formula (3).

Preterm Formula has consistent composition whereas human milk has low and variable nutrient content. This is especially an issue for pHDM as breast milk nutrient content is lower in mothers delivering at full-term compared to preterm, and declines with lactation duration, and because donors have generally delivered at full-term and have been lactating for several weeks. Routine addition of macronutrient fortifier may be necessary but equally may expose babies to excessively high protein intakes, dangerous to neurodevelopment, renal, and metabolic health. In the UK, around half of extremely preterm babies receive fortified feeds (3).

This study will address the longstanding unmet health need of the daily uncertainty faced by healthcare professionals and parents in the UK and around the world around the efficacy and safety of pHDM, Preterm Formula and Fortifier in relation to NEC, survival, and cognitive-language development. Decisions about these feeds affect every extremely preterm baby, every day, in the UK and worldwide. The uncertainties are a major clinical concern and have led to highly variable practice, anxiety for parents, confusion for staff, and risks to patient safety. The study is also needed to establish value for money, given the large cost difference between pHDM (£125-£200 per litre), Preterm Formula (£5 per litre), fortifier (£5/litre of human milk fortified), and the high cost of treating NEC and NEC-related co-morbidities.

1.5 Safety considerations

1.5.1 Clinical concerns

There is fear among clinicians that highly processed cow-milk based products (Preterm Formula and Fortifier) may be damaging to the immature intestinal mucosa and increase the risk of NEC. Evidence to support this fear is however uncertain (see section 1.6 below). Feed intolerance and bowel obstruction from "milk curd" impaction have also been described in relation to the use of fortifiers. Conversely, there is fear that without fortification of human milk, nutrient intake will be inadequate, though evidence to support this concern is also lacking (4).

1.5.2 Adverse neurodevelopment and pHDM

There are emerging safety signals regarding adverse neurodevelopment in relation to use of pHDM. The Canadian Domino Trial randomised very preterm infants pHDM or Preterm Formula to supplement Own Mothers Milk and found higher neuro-impairment at 18 months in the pHDM arm (27.2% v 16.2%; Adjusted Risk Difference 10.6% [95% CI 1.5%, 19.6%]) and a worse mortality/morbidity index (43% v 40%) (5).

In a causal inference analysis of 5 years UK population-based observational data, we found almost 10% lower survival without NEC surgery to 34 weeks gestational age (Adjusted Risk Difference -9.8% [95% CI -11.4, -8.2]) and a surgical NEC rate that was twice as high in very preterm infants receiving supplemental pHDM, compared with Preterm Formula (6). These unexpected, counterintuitive findings may reflect unmeasured confounding (i.e., clinician tendency to use pHDM in infants they consider more at risk of NEC) but nonetheless are also justification for a randomised controlled trial (RCT).

An observational study from the Canadian Neonatal Network of infants born less than 29 weeks gestation, evaluated the relationship between neurodevelopment and enteral feeding (7). Infants that received any formula were excluded. These authors found that for each day of reallocating Own Mother's Milk to pHDM was associated with a statistically significant and highly clinically relevant greater odds of cognitive and language impairment at 18-24 months corrected age. This study indicates not only that pHDM should not be considered equivalent to Own Mother's Milk but additionally may be harmful to neurodevelopment.

1.5.3 Adverse neurodevelopment and routine fortification

When added to human milk, current fortifiers provide about 1.1-2.4g additional protein/100 ml in an appropriate ratio with non-protein energy. Preterm milk protein in the first month averages around 1.5g/100 ml but the range is wide (0.7-2.4 g/100 ml) Given that total feed volumes of 180-200 ml/kg are common in the UK, routine fortification risks providing very high protein intakes, exceeding 5g/kg/day, which may be damaging to neurodevelopment (8). Concerns have been raised that accelerated weight gain from fortification increases the risks of adverse long-term consequences such as obesity, insulin resistance and hypertension (4). The findings of a systematic review and meta-analysis of planned high protein intake after birth for infants born preterm was that this might be harmful for survival, neurodevelopment and metabolism during infancy, and does not improve growth after the neonatal period. The authors concluded that protein intakes ≥3.5 g/kg/d should not be recommended for children born preterm (8).

We performed an analysis of 5 years of whole population data from the UK National Neonatal Research Database (NNRD) in which we compared very preterm infants who had received fortified human milk feeds and those that had not (35,389 infants) (unpublished data). We employed Targeted Maximum Likelihood Estimation, a machine learning approach to address confounding in observational data and maximise causal inferences. We found infants receiving fortified feeds had substantially higher "survival to 34 weeks gestational age without surgical NEC" (Risk Difference 15.1%, 95% CI 14.5 to 15.7). The risk difference increased with decreasing gestational age. "Surgical NEC" was also lower in infants receiving fortified feeds but with a smaller effect size (Risk

Difference -1.4%, 95% CI -1.7 to -1.1). These data may reflect residual confounding but also provide justification for a randomised trial.

1.6 Evidence reviews

We searched the Cochrane Central Register of Controlled Trials for reviews and meta-analyses of published studies, and clinical trial registries for ongoing or recently completed trials (ISRCTN Registry; World Health Organisation International Clinical Trials Registry Platform; ClinicalTrials.gov). Collectively, these show there is inadequate evidence of efficacy and safety for the interventions.

1.6.1 pHDM or preterm formula as supplemental feeds

At initial time of preparing the funding application, the Cochrane systematic review and meta-analysis comparing pHDM and formula as either sole diet or supplement to Own Mother's Milk identified 12 RCT (1879 infants) and included 9 trials (1675 infants) in the NEC analysis (9). No individual trial showed benefit from either pHDM or formula.

Meta-analysis of data from the 9 RCT assessing NEC, in which the comparison combined trials of sole and supplementary diets, showed higher risk with formula (Relative Risk 1.87; 95% Confidence Interval (CI) 1.23, 2.85). However, the Cochrane reviewers sounded several notes of caution. There was no significant reduction in NEC in RCT comparing pHDM and formula as supplement to Own Mother's Milk. Neither sole nor supplementary comparisons show significant differences in outcomes that would be important corroboration of benefit from pHDM (mortality, bloodstream infection, or in the single trial examining this, neurodevelopment). Seven of the RCT took place in the 1970s and 80s when the patient population differed substantially from that of today; sample sizes were inadequate; methodological quality was poor; and medically managed NEC was included in the outcome, which is an imprecise diagnosis highly liable to ascertainment bias. The Cochrane reviewers concluded further research is needed to establish the effects of pHDM or Formula as supplements to Own Mother's Milk.

The same group of authors updated their review in 2024 to include 2261 infants from 11 small trials in the analysis relating to NEC (10). They included two new trials; the Milk Trial (n=483) conducted in the United States, comparing pHDM with Preterm Formula in extremely preterm infants receiving no or minimal Own Mother's Milk (11) and our feasibility study PREMFOOD (NCT01686477) (12). The MILK Trial failed to complete planned recruitment but showed no significant differences in 2-year neurodevelopment (primary outcome), mortality or bloodstream infection. There was lower medical NEC (secondary outcome) in the pHDM arm. Details of surgical NEC were not provided.

In PREMFOOD, we compared fortified human milk feeds (Own Mother's Milk supplemented if required with pHDM), unfortified human milk feeds, and feeds of own Mother's Milk supplemented if required with Preterm Formula. We found no significant between-group differences in growth or body composition at term or term plus 6 weeks. Two infants met rescue criteria for slow weight gain, one receiving exclusive feeds of pHDM, and one exclusive feeds of Own Mother's Milk. There were two deaths, both in infants exclusively fed Own Mother's Milk. Two infants developed NEC; one with surgical NEC had only received Own Mother's Milk and pHDM; one with medical NEC had only received Own Mother's Milk (12).

The Cochrane authors conclude that the risk of NEC is halved through use of pHDM in place of formula (10). However, the diagnosis of NEC in the included studies was very variable and included medical NEC, and there remains no evidence of a reduction in late-onset infection or mortality, which would be important corroboratory evidence. Additionally, no data are presented on surgical NEC.

1.6.2 Routine protein-carbohydrate fortification of human milk

The current Cochrane review and meta-analysis compares fortified and unfortified human milk (18 RCT; 1456 infants) and finds no strong statistical evidence of an effect on NEC (13 RCT; 1110 infants; Relative Risk 1.37 [95% CI 0.72, 2.63]) (13). Only one RCT in this meta-analysis (245 infants), conducted in 1996, assessed neurodevelopment at 18 months (no difference). Fortification increased in-hospital growth but only two RCT, conducted in 1996 and 1998 respectively, assessed growth at 12-18 months (no differences). The trials were generally small and methodologically weak. Of two other relevant Cochrane reviews, one, comparing trials of human milk fortifier providing different amounts of protein (9 RCT; 861 infants), showed small increases in in-hospital weight gain with higher protein intakes (14). The other, comparing early versus late fortification (2 RCT; 237 infants) showed little or no differences in in-hospital growth, surgical NEC, invasive infection, or all- cause mortality (15). Neither reported long term growth or neurodevelopment.

A review of RCT targeting optimal protein delivery identified two studies evaluating neurodevelopmental outcome (16). One randomised <1250g birth weight infants to higher (4.8 g/kg per day) or lower (3.5 g/kg per day) protein intakes for 28 days. Infants receiving the higher intake had better hearing and language scores at 12 and 18 months and greater length gain at 9 months). The other randomised infants <1500 g to 4.2 g/kg per day or 3.6 g/kg per day protein and found no differences in growth or neurodevelopment at 12 and 18 months.

1.7 Justification for the study

This research is needed now because the uncertainties have led to current practice that is highly variable, and a proliferation of non-evidenced based guidelines. Parents, patients (those living with the long-term consequences of neonatal conditions), clinicians, and the James Lind Priority Setting Partnership have repeatedly ranked identification of enteral feeding strategies that reduce NEC a priority for well over a decade (17-19).

The NICE guidance "*Donor milk banks: service operation*" acknowledges the limited high-quality evidence for use of pHDM and includes the recommendation for research to evaluate efficacy in improving outcomes and identify babies who would benefit most (20). The 2023 British Association of Perinatal Medicine "*Framework for use of Donor Milk*" also highlights the urgent need for this research (21). The American Academy of Pediatrics and European Society for Paediatric Gastroenterology, Hepatology and Nutrition only conditionally support use of pHDM as the supplementary feed of choice, acknowledging the benefits of Own Mother's milk over pHDM, the variable nutrient content and uncertain non-nutrient benefits of pHDM, and the limited evidence-base for practice.

2 OBJECTIVES AND ENDPOINTS

2.1 Primary objectives

- To assess, in babies born <29 weeks gestation, the efficacy of pHDM compared with Preterm Formula when used as a supplement should there be insufficient milk from their own mother (Own Mother's Milk) on "survival to 34 weeks postmenstrual age without surgical NEC"
- To assess, in babies born <29 weeks gestation, if routine cow-milk based protein-carbohydrate fortification of human milk feeds (Own Mother's Milk and pHDM) compared with no routine fortification, affects "survival to 34 weeks postmenstrual age without surgical NEC"

2.2 Secondary objectives

- To assess the efficacy of pHDM compared with Preterm Formula on language and cognitive development at age 2-years, and other outcomes in babies born <29 weeks gestation, when used as a supplement should there be insufficient milk from their own mother (Own Mother's Milk)
- To assess if routine cow-milk based protein-carbohydrate fortification of human milk feeds (Own Mother's Milk and pHDM) in babies born <29 weeks gestation affects language and cognitive development at age 2-years, and other outcomes

2.3 Tertiary objectives

- To determine if pHDM and Preterm Formula exert different effects on neurodevelopment through the mechanism of altered cerebral white matter microstructure.
- To establish if the additional cost of pHDM to the NHS is justified through a reduction in NEC
- To establish if the additional cost of fortifier, generates savings through improved survival to age two-years corrected for prematurity without moderate-severe cognitive-language impairment
- To collect and store faecal samples from participants recruited in Edinburgh who are enrolled in randomisation 1, for future mechanistic studies subject to funding

2.4 Endpoints

2.4.1 Primary

Assessed at 34 weeks postmenstrual age

- Survival without surgical NEC

2.4.2 Secondary

Assessed at 34 weeks postmenstrual age

- Survival
- Surgical NEC
- Spontaneous Intestinal Perforation

Assessed at 36 weeks postmenstrual age

- Bronchopulmonary dysplasia

Assessed at postnatal age 28 days

- Survival

Assessed at neonatal unit discharge (or death)

- Survival
- Surgery for NEC or NEC related condition after 34 weeks postmenstrual age
- Medical NEC
- Age in days to achieve an enteral intake of 150 ml/kg/day
- Treated retinopathy of prematurity

- Severe brain injury
- Any diagnosis of milk-curd obstruction
- Length of neonatal unit stay
- Number of episodes of bacterial or fungal bloodstream infection
- Number of episodes of bacterial or fungal cerebrospinal fluid infection
- Number of episodes of bacterial or fungal urinary tract infection
- Number of days of antibiotic treatment
- Number of days on parenteral nutrition
- Number of days nil by mouth
- Weight, length, and head circumference Z-scores
- Change from birth in Z-scores for weight, length, and head circumference
- Any breast feeding (suckling at breast)
- Exclusive breast feeding (suckling at breast)
- Receiving any expressed Own Mother's Milk
- Receiving exclusive expressed Own Mother's Milk
- Maximum serum urea, creatine, and alkaline phosphatase
- Health resource use

Assessed at neonatal unit discharge (or death) in babies with NEC surgery

- Drain insertion prior to surgery (yes/no)
- Diagnosis of short bowel syndrome (yes/no)
- Diagnosis of intestinal failure-associated liver disease (yes/no)
- Length of bowel resected (cm)
- Primary surgical procedure (ileostomy; colostomy; end-to-end anastomosis)
- Number of re-operations (excluding primary operation)

Assessed at age 2-years corrected for prematurity

- Survival without moderate-severe cognitive-language impairment
- Survival
- Moderate-severe cognitive-language impairment
- Cognitive sub-score
- Language sub-score
- Gross motor function
- Hearing impairment
- Vison impairment

2.4.3 Tertiary

Assessed at term-equivalent age (37-44 weeks postmenstrual age)

- Cerebral white matter microstructure indexed by fractional anisotropy values using tract-based spatial statistics

Assessed at neonatal unit discharge (or death)

- In-hospital healthcare costs

Assessed at age two-years (corrected for prematurity)

- Healthcare costs (subject to additional funding)

2.4.4 Endpoint selection

Endpoints have been selected taking core neonatal outcome sets into account (22, 23).

2.5 Summary table of objectives and endpoints

Objectives	Endpoints	Timepoints of evaluation of endpoints
Primary		
• To assess. in babies born <29 weeks gestation, the efficacy of pHDM compared with Preterm Formula when used as a supplement should there be insufficient milk from their own mother (Own Mother's Milk) on "survival to 34 weeks postmenstrual age without surgical NEC", • To assess, in babies born <29 weeks gestation, if routine cow-milk based protein-carbohydrate fortification of human milk feeds (Own Mother's Milk and pHDM) compared with no routine fortification, affects "survival to 34 weeks postmenstrual age without surgical NEC"	Survival without surgical NEC	34 weeks postmenstrual age
Secondary		
• To assess the efficacy of pHDM compared with Preterm Formula on language and cognitive development at age 2-years, and other outcomes in babies born <29 weeks gestation, when used as a supplement should there be insufficient milk from their own mother (Own Mother's Milk)	• Survival • Surgical NEC • Spontaneous Intestinal Perforation	34 weeks postmenstrual age
	• Bronchopulmonary dysplasia	36 weeks postmenstrual age

• To assess if routine cow-milk based protein-carbohydrate fortification of human milk feeds (Own Mother's Milk and pHDM) in babies born <29 weeks gestation affects language and cognitive development at age 2-years corrected for preterm birth, and other outcomes		
	• Survival	Age 28 days
	• Survival • Surgery for NEC or NEC related condition after 34 weeks postmenstrual age • Medical NEC • Age in days to achieve an enteral intake of 150 ml/kg/day • Treated retinopathy of prematurity • Severe brain injury • Any diagnosis of milk-curd obstruction • Length of neonatal unit stay • Number of episodes of bacterial or fungal bloodstream infection • Number of episodes of bacterial or fungal cerebrospinal fluid infection • Number of episodes of bacterial or fungal urinary tract infection • Number of days of antibiotic treatment • Number of days on parenteral nutrition • Number of days nil by mouth • Weight, length, and head circumference Z-scores • Change from birth in Z-scores for weight, length, and head circumference • Any breast feeding (suckling at breast) • Exclusive breast feeding (suckling at breast) • Receiving any expressed Own Mother's Milk • Receiving exclusive expressed Own Mother's Milk • Maximum serum urea, creatine, and alkaline phosphatase • Health resource use	Neonatal unit discharge (or death)

	• Survival without moderate-severe cognitive-language impairment • Survival • Moderate-severe cognitive-language impairment • Cognitive sub-score • Language sub-score • Gross motor function • Hearing impairment • Vison impairment	Age 2-years corrected for preterm birth
In babies with NEC surgery	• Drain insertion prior to surgery • Diagnosis of short bowel syndrome • Diagnosis of intestinal failure-associated liver disease • Length of bowel resected (cm) • Primary surgical procedure (ileostomy; colostomy; end-to-end anastomosis)	Neonatal unit discharge (or death)
In babies with NEC surgery	• Number of re-operations (excluding primary operation)	Age 2-years corrected for preterm birth
Tertiary		
• To determine if pHDM and Preterm Formula exert different effects on neurodevelopment through the mechanism of altered cerebral white matter microstructure	• Cerebral white matter microstructure	Term equivalent age (37-44 weeks postmenstrual age)

• To establish if the additional cost of pHDM to the NHS is justified through a reduction in NEC	• Health resource use	Neonatal unit discharge (or death)
• To establish if the additional cost of fortifier, generates savings through improved survival to age 2-years corrected for preterm birth without moderate-severe cognitive-language impairment	• Health resource use (subject to future funding)	Age 2-years corrected for preterm birth
• To collect and store faecal samples from participants recruited in Edinburgh who are enrolled in randomisation 1, for future mechanistic studies	• N/A subject to future funding	N/A

3 STUDY DESIGN

3.1 Design

An efficient, UK-wide, adaptive, real-world-data-enabled, digital technology-facilitated, multicentre, 2-randomisation, open-label, controlled trial embedded in routine clinical care. The study includes an internal pilot to assess rates of recruitment, co-randomisation, and withdrawal of consent, and includes formal interim efficacy monitoring. See Study Flow Chart, Appendix 1.

The study involves two randomisations. In randomisation 1, we will compare the benefits of supplementation with either pHDM or Preterm Formula if the available volume of Own Mother's Milk is insufficient to meet her baby's needs. In randomisation 2 we will assess the benefits of routine versus no routine fortification of human milk; rescue fortification is permitted at the discretion of the attending clinician if a baby meets pre-defined criteria (see section 5.6.2).

3.2 Mechanistic study

3.2.1 Encephalopathy of prematurity

COLLABORATE includes an embedded mechanistic sub-study led by Co-I Boardman that will use magnetic resonance imaging (MRI) to determine whether pHDM and Preterm Formula have different effects on cerebral white matter maturation, the hallmark feature of encephalopathy of prematurity, and hence impact the development of brain networks that underpin cognition. We will explore the potential differential effects of the interventions on cortical morphology and microstructure. This sub-study will involve 120 infants participating in COLLABORATE from the Royal Infirmary of Edinburgh. They will undergo 3T brain MRI using a research-dedicated, Wellcome-funded, brain-optimised Siemens MAGNETOM Prisma 3T scanner and 16-channel phased-array paediatric head and neck coil.

3.2.2 Gut microbiota

The gut microbiota is thought to play a role in human health and disease, including child development (24); it is modified by age at birth, sex, mode of delivery, antibiotic exposure, and feed type. The microbiome may mediate interactions of the gut-brain axis, including in preterm infants (25-27). For infants enrolled in the mechanistic study in Edinburgh, faecal samples will be collected at enrolment and weekly to 34 weeks. Samples will be processed and stored at -80°C in the Centre for Reproductive Health at the University of Edinburgh for later analyses subject to ethics approval and funding, exploring mechanisms linking nutritional exposures to brain development through gut-brain interactions.

3.3 Cost-effectiveness study

COLLABORATE embeds a within-trial economic evaluation, led by Co-I Luengo-Fernandez, to establish value for money of pHDM versus preterm formula, due to the large cost difference between the two interventions (up to £200 per litre for pHDM versus £5 per litre for formula) and the high costs of NEC treatment. The principal cost-effectiveness hypothesis for randomisation 2 (which will be subject to additional funding) is: In babies born <29 weeks gestation (P) who receive routine (I) versus no routine (C) fortification of human milk feeds, the additional cost of fortifier, generates savings through improved survival to age two-years corrected for prematurity without moderate-severe cognitive-language impairment (PO).

4 STUDY SETTING AND PARTICIPANTS

4.1 Study setting

This study will recruit from approximately 80 Neonatal Intensive Care Units and Local Neonatal Units in England, Scotland, Wales, and Northern Ireland. As per standard practice for neonatal care across the UK, all other units across the country will be opened as continuing care sites (up to approximately 190 sites in total).

4.2 Inclusion and exclusion criteria

4.2.1 Randomisation 1

4.2.1.1 Inclusion criteria

- Born <29 weeks gestation
- No condition precluding enteral feeding
- Maternal intention to express breast milk
- Parent verbal consent

4.2.1.2 Exclusion criteria

- Baby has already received pHDM or Preterm Formula

4.2.2 Randomisation 2

4.2.2.1 Inclusion criteria

- Born <29 weeks gestation
- No condition precluding enteral feeding
- Maternal intention to express breast milk
- Parent verbal consent

4.2.2.2 Exclusion criteria

- Baby has already received Fortifier

4.2.3 Mechanistic sub-study

4.2.3.1 Inclusion criteria

- Participants in randomisation 1 recruited at the Royal Infirmary of Edinburgh
- Written parent consent

4.2.3.2 Exclusion criteria (MRI)

- Infants with congenital anomalies: structural or functional (e.g., metabolic disorders) that occur during intrauterine life and can be identified prenatally, at birth or later in life (WHO definition)
- Infants with a contraindication to MRI at 3Tesla

4.2.3.3 Exclusion criteria (stool sample collection)

- None

4.3 Planned recruitment rate

There are currently 181 neonatal units in the UK; 55 are designated Neonatal Intensive Care Units (NICU) providing the highest level of care; 82 are Local Neonatal Units (LNU) providing intermediate care; and 44 are Special Care Baby Units (SCBU).

We aim to recruit from approximately 80 NICU and LNU, with the remaining units, including all SCBU, participating as step down sites which will continue randomised treatments and contribute outcome data. We will focus on maximising NICU participation as they admit about 70% of infants <29w gestation. We plan to open sites over the first 6 months. We based the recruitment site figure on a conservative assumption of 60% of NICU and LNU participation (i.e. 80 sites).

5 PROCEDURES AND MEASUREMENTS

5.1 Identification and recruitment of participants

Babies will be assessed for eligibility by a member of the clinical care team (an appropriately trained and experienced neonatal doctor or nurse) after admission to the neonatal unit. Parents must not have opted-out of their baby's data being included in the NNRD.

Cot cards and stickers for paper medical notes will be used to remind staff and parents about participation and to which intervention/s the baby is randomised. Cot cards and stickers will also contain a QR code which links to information on the COLLABORATE study website.

If electronic notes are used, a flag should be added to the participants record to identify their participation in the study.

5.2 Consent

5.2.1 Type and timing of consent

5.2.1.1 Main study

As this study compares interventions that are already in widespread use, verbal consent will be sought for the main study. This will be documented in the eCRF and medical/research records by the member of the clinical care team who has explained the study and sought consent. Verbal consent is well received by parents and clinicians and has been used in other RCT evaluating interventions that are already in wide use, including the current NIHR funded clinical trial of a medicinal product, BASE (ISRCTN 18260410).

The verbal consent statement will be completed by the participating site staff electronically in OpenClinica to aid central monitoring of consent data. A paper back-up version may be used where access to the OpenClinica database is not possible.

Participants will be provided with a copy of the Participant Information Sheet and completed verbal consent statement. Consent will be sought a time considered clinically appropriate and before the baby has received any supplemental feeds or fortifier.

Before discharge from the neonatal unit, a discussion will take place with parents to remind them that the trial team will contact them at 2 years to ask them to complete the Parent Report of Children's Abilities-Revised (PARCA-R) questionnaire. At discharge, parents will be asked to confirm their contact details are up to date to improve data return rates.

5.2.1.2 Mechanistic sub-study

Parents will be approached to tell them about the MRI procedure and stool sample collection at a clinically appropriate time, and their written informed consent sought for their baby to take part. This is an optional sub-study, and not all babies recruited in the participating centre will take part.

5.2.2 Consent sought

5.2.2.1 For all participants

Verbal consent will be sought for:

- Randomisation 1
- Randomisation 2
- The results of the age 2-year Parent Report of Children's Abilities-Revised (PARCA-R) assessment to be included in the infant's National Neonatal Research Database record and hence available for other approved research studies
- Longer term follow-up using linkage of other routinely collected NHS and non-NHS data (e.g., Hospital Episodes Statistics; National Pupil Database) using the NHS number and other identifiers (subject to additional funding)
- Permission to forward study results to participating families
- Permission to use data obtained in COLLABORATE in future approved research studies
- Permission to recontact parent/guardians for future research

5.2.2.2 For babies participating in the mechanistic sub-study

Additional written consent will be sought for**:**

- Magnetic resonance imaging at term equivalent age (37-44 weeks)
- Permission to share anonymised MRI data with other researchers running research ethics approved studies subject to data access and collaboration arrangements governed by the University of Edinburgh
- Permission to collect weekly stool samples from birth to neonatal unit discharge and at time of MRI scan
- Permission to inform the General Practitioner of participation in COLLABORATE mechanistic sub- study
- Permission to use stool samples obtained in future approved research studies

5.3 Randomisation: timing and method

5.3.1 Timing

Babies may participate in either or both randomisations; see Flow Chart, Appendix 1.

Randomisation 1 to pHDM or Preterm Formula, will occur when the attending clinician decides a supplemental feed is required because the volume of Own Mother's Milk is insufficient.

Randomisation 2, to routine fortification or no routine fortification, will occur when the baby is receiving between 60-120 ml/kg/day of human milk feeds (Own Mother's Milk and/or pHDM). By "fortification" we mean immediate or incremental increase to "full strength fortification" according to neonatal unit practice. We refer to "no routine fortification" because rescue fortification is permitted (see section 5.6.2).

5.3.2 Method

Randomisation will be carried out electronically using an on-line system managed by the ICTU Clinical Data Systems Team. We will use minimisation (with 10% random allocation) to achieve

balance between the number of babies in each treatment group over important, well-recognised prognostic factors [neonatal network (N=13); gestational age category (<26w; >/=26w); severe growth restriction (<10% centile; >/=10% centile based on standard criteria)]. Multiple births will be randomised individually in keeping with our feasibility study (12), other feeding trials (28), and parent focus group advice.

5.4 Procedures and evaluations

There will be no research-related procedures or evaluations except for infants participating in: the embedded mechanistic sub-study, who will be invited for brain MRI which is a well-established procedure and from whom serial stool samples will be obtained.

5.5 Schedule of assessments

Timepoint → **Assessment ↓**	**On admission to neonatal care**	**28 days postnatal age**	**34 weeks postmenstrual age**	**36 weeks postmenstrual age**	**Discharge**	**37-44 weeks postmenstrual age**	**2 years corrected for prematurity** *(23.5–27.5 months)*
Eligibility	X						
Consent	X						
Randomisation*	X						
Highest recorded serum urea, creatinine, alkaline phosphatase					X		
Clinical endpoints from NNRD data		X	X	X	X		
Additional clinician recorded endpoints					X		
MRI**						X	
Stool sample**	X	X	X			X	
PARCA-R***							X
Adverse Reactions		X	X		X	X	

** When the participant meets the relevant inclusion/exclusion criteria for each randomisation*

*** Only participants in the mechanistic sub-study*

**** To be completed using the digital PARCA-R where possible (facilitated through ICTU)*

5.6 Safety considerations

5.6.1 Nutritional supplements

We recommend that infants randomised to the “no routine fortification” arm receive routine oral phosphate supplements to protect against metabolic bone disease. Other supplements including vitamins to be provided in accordance with the policies of the participating neonatal unit.

5.6.2 Faltering growth

Clinicians may consider discretionary use of fortifier in babies participating in randomisation 2 should there be evidence of growth faltering and non-nutritional causes have been excluded; guidance will be provided in the study manual.

Clinicians considering rescue fortification are advised to discuss this with the clinical CI or delegate. Rescue fortification will be documented in the eCRF and factored into analyses.

5.6.3 MRI

The MRI scanner makes a loud knocking noise, so earmuffs are used to prevent noise discomfort and encourage infants to sleep. We will use established procedures that ensure infant safety and physiological stability during imaging (29). The infant will have continuous monitoring of vital signs (heart rate and oxygen saturation) with an electronic monitor. The attending clinician will record observations every 5 minutes until 1 hour after the infant has woken up, and the scan will be stopped if there are any abnormalities in monitoring. Full neonatal resuscitation facilities are available on site. Standard Operating Procedures for ensuring safety in the MRI environment in place in the Edinburgh Imaging facility will be followed.

5.7 Follow-up

No research-related follow-up visits are required. Age 2-year cognitive and language outcomes will be obtained using the parent-completed PARCA-R in accordance with NICE recommendation (30). A digital version of the PARCA-R will be made available to clinical teams should they wish to use this in which case parents will be registered to do so at neonatal unit discharge (31).

5.8 Laboratory evaluations

No research-related laboratory evaluations are required for the majority of participants. Maximum serum urea, creatine, and alkaline phosphatase will be recorded from routine blood results during time on the neonatal unit.

For participants recruited at the Royal Infirmary of Edinburgh, consent will be sought for collection of serial stool samples at enrolment and each week until 34 weeks postmenstrual age. An additional sample will be collected at the time of the MRI scan if the baby is enrolled to both aspects of the mechanistic sub-study. Between 7 and 14 stool samples will be collected depending on birth gestational age.

5.9 Incidental findings

Incidental findings relate only to the MRI scans.

All MRI scans are acquired using a brain optimised research scanner within a tight quality-controlled environment at Edinburgh Imaging. Neuroimaging data will be obtained by brain research radiographers and subject to Edinburgh Imaging Quality Control procedures. Structural MRI data are reported according to a structured report by a paediatric radiologist, including incidental findings (29). We will notify the child’s general practitioner of any incidental findings that

may be clinically actionable and any relevant NHS services that have been involved, through the clinical care team.

5.10 Permanent discontinuation of study interventions

Participant parents/guardians may discontinue study interventions by request. The attending clinician may discontinue participation if it is considered, following discussion with the Chief Investigator or authorised delegate**,** that the participant's health would be compromised by continuation. The reason for discontinuation will be recorded in the eCRF and medical records. Participants may also discontinue study interventions if the funder, sponsor or independent committees request this.

The participants will continue to be followed up even if the intervention has been discontinued

5.11 Withdrawal from the study

Withdrawal from the study refers to withdrawal from all study treatment and study procedures and can occur for the following reasons:

- Participant's parent or legal guardian's decision
- Participant is lost to follow-up
- Investigator's decision

As specified in the Parent Information Leaflet, should the parent/legal guardian withdraw consent for their baby's participation, data already available in the NNRD will be utilised.

6 SAFETY REPORTING

6.1 Adverse Events (AE)

An AE is any untoward medical occurrence in a clinical trial participant. An AE can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), whether or not considered related to the trial protocol.

6.2 Serious Adverse Events (SAE)

An SAE is defined as any event that

- Results in death
- Is life-threatening*
- Requires prolongation of hospitalisation**
- Results in persistent or significant disability or incapacity

* "Life-threatening" in the definition of "serious" refers to an event in which the participant 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.

** "Hospitalisation" means any extension of hospitalisation; it does not apply to scheduled admissions that were planned before study inclusion or visits to casualty (without admission).

Medical judgement will be exercised in deciding whether an adverse event/reaction is serious. Important adverse events/reactions that are not immediately life-threatening, or do not result in death or hospitalisation but may jeopardise a participant or may require intervention to prevent one of the other outcomes listed in the definition above should also be considered serious.

6.3 Adverse Reactions (AR)

All untoward and unintended responses to the study intervention. All AE judged by either the reporting investigator or the sponsor as having reasonable causal relationship to the study intervention qualify as AR.

6.4 Serious Adverse Reactions (SAR)

A SAR is defined as a SAE that is judged to be related to any study intervention given to the participant.

6.5 Severity of adverse events

Mild:	Appears easily tolerated
Moderate:	Discomfort to cause interference with usual behaviour
Severe:	Not demonstrating usual activity

6.6 Causality of adverse events

The assignment of causality for AE should be made by the investigator or delegate responsible for the care of the participant using the definitions in the table below.

If any doubt about the causality exists, the local investigator should inform the study coordination centre who will notify the Chief Investigator. Other clinicians may be asked to advise. In the case of discrepant views on causality between the investigator and others, all parties will discuss the case.

Unrelated:	No evidence of any causal relationship
Unlikely:	Little evidence to suggest there is a causal relationship (e.g. the event did not occur within a reasonable time after the trial intervention). There is another reasonable explanation for the event (e.g. the participant's clinical condition, other concomitant treatment)
Possible:	There is some evidence to suggest a causal relationship (e.g. because the event occurs within a reasonable time after the trial intervention). However, the influence of other factors may have contributed to the event (e.g. the participant's clinical condition, other concomitant treatments)
Probable:	There is evidence to suggest a causal relationship and the influence of other factors is unlikely
Definite:	There is clear evidence to suggest a causal relationship and other possible contributing factors can be ruled out

6.7 Adverse reaction recording

The safety reporting window for this trial will be from randomisation (1 or 2) until 34 weeks PMA, discharge from neonatal care, or death, whichever is sooner. The reporting timelines are the same for infants participating in the mechanistic sub-study. Sites will be trained in safety reporting requirements.

Active monitoring of participants after the end of the trial is not required but if the investigator becomes aware of safety information that appears to be related to the trial, after a participant has completed the study, this should be reported to the Sponsor's delegate (ICTU) at collaborate@imperial.ac.uk.

6.8 Adverse reaction reporting

The use of pHDM, Preterm Formula and fortifier are established in routine care. In this extremely preterm population, we anticipate frequent foreseeable AE, SAE and AR, and a mortality rate to discharge from neonatal care of around 10%. For these reasons, AE, SAE, and AR will not be recorded.

All expected SAR have been listed as study endpoints, hence these events will be excluded from safety reporting. Any other SAR will be **unexpected** and extremely rare. These **unexpected** events will be reported to the Sponsor and Ethics Committee within 15 days of the investigator becoming aware of the event.

Cumulative data for safety endpoints (including expected SAR) will be included in regular safety reports reviewed by the Data Monitoring and Ethics Committee in accordance with the charter. Due to the established use of all trial interventions, additional safety oversight of expected events is not required.

Reporting of unexpected SAR will be performed through the eCRF. Unexpected SAR should be reported within 24 hours of the site study team becoming aware of the event as serious, unexpected and related (as described in section 6.4 and 6.6). SAR will be reported to the Sponsor's delegate (ICTU). Further details can be found in the safety reporting manual.

6.9 Reporting urgent safety measures

If any urgent safety measures are taken the Chief Investigator or authorised delegate will immediately and, in any event, no later than 3 days from the date the measures are taken, give written notice to the relevant REC of the measures and the circumstances giving rise to them.

7 STATISTICAL ANALYSES

7.1 Statistical Analysis Plan

We will construct a detailed Statistical Analysis Plan, signed off by the Chief Investigators and Trial Steering Committee prior to the first interim analysis. We will summarise baseline characteristics by randomisation arm within each comparison using appropriate statistical measures (mean, standard deviation; median, interquartile range; frequencies; percentages). The flow of participants through both comparisons will be reported according to the CONSORT Extension for Adaptive Designs (32). We will target a treatment policy estimand to estimate the effect of each intervention as delivered in practice regardless of any events that occur post-randomisation. The efficacy and safety analyses for both randomisations will be based on the intention-to-treat population. Missing data is expected to be very low as this is retrieved from NNRD. It is specified in the Parent Information Leaflet that should they withdraw consent for their baby's participation, data already available in the NNRD will be utilised.

7.2 Sample size and power

7.2.1 Main study

This is a group-sequential trial with 2 interim and 1 final analysis (i.e., an adaptive trial), hence the sample size is not fixed. The maximum sample size is 3252, (n=2168 in each randomisation; n=1084 per arm) assuming no early stopping, estimated on the composite primary efficacy outcome (survival to 34 weeks gestational age without surgical NEC (Y/N)).

For the randomisation 1 (pHDM vs Formula supplement) an observational analysis of NNRD data estimated the difference in the proportion surviving to 34w PMA for babies born <29 weeks' gestation to vary between 0.18 and 0.52 depending on week of gestational age (favouring supplementation with preterm formula) (6) For the sample size calculation we targeted a highly conservative MCID in this proportion of 0.05 based on a baseline of 0.82 which we accurately estimated from the NNRD for this population of babies in the UK (i.e. from 0.82 to 0.87).

For randomisation, 2 (fortifier vs no routine fortifier) an unpublished observational analysis from NNRD data estimated the difference in proportion data surviving to 34w PMA for babies born <29 weeks gestation to vary between 0.06 and 0.75 depending on week of gestational age (favouring fortifier). Taking a conservative approach and in line with the first comparison, we have chosen to target the same MCID of 0.05.

We have calculated power based on a fixed sample size of 3252 unique babies using rpact (33) where interim timings were chosen to minimise the expected sample size. We anticipate at least 50% of babies will be co-enrolled into both randomisations. If co-enrolment is higher, power will increase. We have used an O'Brien & Fleming design with two-sided overall significance level of 5% to account for two interims at 50% and 75% and a normal approximation assumption for rates. For both randomisations we have factored in 2% missing outcome data due to carers withdrawing consent from routine data collection in NNRD.

Based on all the above assumptions, n=3252 (n=2168 in each randomisation and n= 1084 per arm) will achieve a minimum of 87% power for each randomisation. The expected sample size across for each randomisation is n=2439.

7.2.2 Mechanistic sub-study

Based on computational modelling and prior precedent from neonatal neuroprotection trials, a study of 60 infants in each treatment group will enable detection of a 10% difference in fractional anisotropy with 80% power, 2-sided 5% significance (34).

7.3 Primary endpoint analysis

We will model the main intervention effect for each randomisation separately. For both, we will use a binomial Generalized Estimating Equation (GEE) model with identity link and a log link function to estimate adjusted risk difference and adjusted risk ratio (in line with CONSORT reporting recommendations) with robust variance. This will enable us to account for within network correlations. Intervention effect for the first and second randomisation will be modelled separately. Both models will be adjusted for minimisation variables.

The analysis model for randomisation 1 will include whether or not an infant received fortification (Y/N) and whether or not they were randomised to this (randomisation 2; Y/N)). Though randomisation 2 will occur after randomisation 1, they are close in time and receiving fortification may be more likely to be a predictor of the primary outcome than a result of taking part in randomisation 1. Therefore, randomisation 2 and receipt of fortification will be treated as baseline covariates.

The analysis model for randomisation 2 will include adjustment for taking part in randomisation 1 (Y/N) and type of supplementary feed received (pHDM or formula). A supplementary analysis for randomisation 2 will be performed to determine the effect of rescue fortification in the standard of care arm (no routine fortification) will be undertake using principal stratum and complier average causal effect approaches (no rescue fortification is defined as a complier in the standard of care arm). In the unlikely case that missing data are greater than 2%, a sensitivity analysis will be performed using multiple imputation.

Binary secondary and key safety outcomes (death, surgical NEC, medical NEC, infection) will be analysed using the same principled approach as the primary outcome. The age 2-year PARCA-R will also be evaluated as a continuous outcome and the between arm mean difference estimated using the GEE model with normal distribution and identity link function adjusting for minimisation variables.

7.4 Interim assessment of efficacy

We will conduct two pre-planned formal interim efficacy analyses when 50% and 75% of participants reach 34 weeks gestational age (i.e., primary outcome is available). This analysis will be undertaken by an independent ICTU statistician. Recruitment will continue during interim analyses. The analysis will employ two-sided O'Brien-Fleming efficacy boundaries. Unadjusted between arm differences in proportions will be calculated using the primary analysis models described for the final analysis. An identify link function will be used to obtain adjusted risk differences and corresponding Z-scores. Superiority will be assessed at each stage based on the critical value thresholds (Z-scores) and numbers of participants**.**

Proportion recruited	50%	75%	100%
Randomisation 1, n	954	1431	1908
Randomisation 2, n	1063	1594	2126
Critical value thresholds (Z-scale)	2.863	2.337	2.024

7.5 Subgroup analyses

We will perform subgroup analyses for the primary outcome by gestational age category (<=26 weeks; >26 weeks) and growth centile (<10% centile; >=10% centile). The subgroups will be examined through use of an interaction term between the subgroup and the intervention arm. Results will be judged at an alpha of 0.20, presented as forest plots, and will be hypothesis generating.

7.6 Exploratory analyses

Exploratory analyses will be undertaken to estimate how the volume of supplementary feed (pHDM or formula) and the time of introduction impacts outcome. This will be performed using Parametric G-formula, a method to estimate the effects of treatment strategies adjusting for time-varying confounders affected by prior exposures.

7.7 Safety analyses

We will tabulate adverse events by intervention arms for each randomisation. For safety events of special interest (death, surgical NEC, medical NEC, infection) we will follow the principle of the primary analysis. For all other events we will calculate the incidence rate ratio and present these graphically using dot plots.

7.8 Mechanistic sub-study

We will prepare a mechanistic Statistical Analysis Plan prior to any data analyses. We will use Tract-based Spatial Statistics (TBSS) optimised for neonates to compare voxel-wise statistics

across the white matter skeleton between treatment groups using a general linear model adjusting for gestational age at birth, age at image acquisition, and any important covariates that remain imbalanced after randomisation (35,36). Significance will be set as $p<0{\cdot}05$, following family-wise error rate correction and threshold-free cluster enhancement. TBSS results will be reported as maps overlaying the skeleton where voxels with *p*-values <0.05 are highlighted using a colour bar to show the range of significant values.

Participants will be excluded from TBSS analyses if i) there is a parenchymal injury or brain abnormality identified on structural MRI by clinical reviewers considered likely to confound image registration; ii) if there is no diffusion MRI, or excessive motion; iii) if more than 10 diffusion weighted MRI volumes had slice dropout; or iv) if registration fails. Application of these exclusion criteria will be blind to treatment group allocation to reduce the risk of selection bias. Image analysts will be blind to treatment group allocation until analyses are completed.

Diffusion tensor imaging (DTI) data will be analysed using Functional MRI of the Brain Software Library (37) and DTI-ToolKit. Data will be corrected for phase encoding distortions, eddy-induced distortions and motion using the topup-eddy algorithm, using T2 structural volumes rigidly registered to b0 maps and assuming a bandwidth of zero (no phase-encoding) (38). A diffusion tensor model will be fitted to each voxel and the eigenvalues and eigenvectors used to convert the corrected diffusion weighted images into diffusion tensor volumes.

Tensor-based image registration will be used to produce a population-specific diffusion tensor template. From this template the mean Fractional Anisotropy (FA) volume is derived and thinned by perpendicular non-maximum suppression to create the mean white matter tract skeleton, thresholded at $FA > 0{\cdot}15$ to exclude peripheral tracts. All participants' diffusion tensor volumes will be registered to the diffusion tensor template and FA and other parameters extracted and projected onto the white matter tract skeleton.

7.9 Cost effectiveness study analyses

As COLLABORATE will be viewed as providing definitive evidence by the medical community, there is a pressing need to establish the value for money of pHDM versus preterm formula, especially due to the large cost difference between the two interventions and the high costs of NEC treatment (39). NHS secondary care utilisation from randomisation until neonatal unit discharge will be obtained from the NNRD, as used in other successful economic evaluations (40, 41).

To calculate the costs associated with daily neonatal care received by each baby, we will multiply the number of days spent receiving each level of care, by level-specific national average bed day costs sourced from National Schedule of NHS Costs (42). Such costs are estimated by assigning each level of care a healthcare resource group (HRG) code. HRG are groupings of clinically meaningful activities made primarily on the basis of diagnosis and procedure codes, and within the NHS, are the 'units' of healthcare for which providers receive payment. Costs assigned to each HRG are based on nationally estimated reference costs developed into adequately cover the cost of providing high-quality and cost-effective care. For this analysis, the neonatal bed day costs use will be intensive care (HRG XA01Z), high dependency care (HRG XA 02Z), special care without carer resident alongside baby (HRG XA03Z), special care with carer resident alongside baby (HRG XA04Z) and normal care (HRG XA05Z).

Given that some major clinical activities are not included in these neonatal care HRG we will identify high-cost non-routine procedures captured within the NNRD, considered to be important in this particular population. For example, types of neonatal surgery will be identified from their corresponding Operating Procedure Code Standard and International Statistical Classification of Diseases and Health Related Problems (ICD-10) codes for costing purposes and unit costs obtained from the NHS National Schedule of Costs.

For both trial comparisons, cost results will be reported as means with standard deviations. Mean differences, together with 95% confidence intervals, will be estimated using multilevel generalised gamma linear models with a random intercept at the neonatal unit level. For comparison 2 (fortification versus no fortification), results will additionally be presented by subgroup: no clinical need for supplementation of mother's own milk; and if supplementation is required, by randomisation group in comparison 1, i.e. pHDM and Preterm Formula. Costs will be presented alongside the key primary and secondary outcomes by intervention arm in both comparisons with associated uncertainty. We will use the NNRD to determine the number of extremely preterm babies and hence estimate the NHS budget implications for the UK of all study interventions. This approach will enable various stakeholders (e.g. parents, clinicians and commissioners) to contemplate the impact of pHDM and milk fortification on the outcomes of most relevance to them.

If trial interventions result in better 2-year cognitive-language outcomes, we will develop a decision analytical model to estimate the cost-effectiveness of the intervention up to 18 years of age. This analysis will be conducted from an NHS and societal perspective and the main outcome measure in the economic evaluation will be the child's quality-adjusted life years (QALY). A Markov model will be constructed representing the natural history of infants to extrapolate the within-trial cost-effectiveness results using annual cycles. The structure of the model will be established and agreed within the research team. Observed outcomes and health care resource utilisation for randomised infants will be used to inform the characteristics of a hypothetical cohort entering the model. Transition probabilities indicating movement across health states during the first two years will be obtained from the trial, whereas transition probabilities after the second year will be informed through literature searches. Health care costs and health-related quality of life estimates incurred annually in each health state after neonatal care discharge will be obtained from the literature. Data on informal care and impact on productivity of parents to inform cost parameters in the model will also be obtained from the literature if available.

To be able to follow-up trial participants using routine sources, we will seek parent consent to access long-term data. This will enable future data linkage to the Department for Education National Pupil Database on performance in Key Stage 1 and Special Educational Needs, and use of health services and mortality through Hospital Episode Statistics.

7.10 Internal pilot

We will run an internal pilot to assess recruitment rate; co- enrolment rate; and withdrawal of consent rate. The internal pilot will run for 9 months (6-15 months).

We need adequate recruitment for each of the comparisons to be powered to detect the MCID of 0.05. We estimate recruitment will increase over the first 9 months from 25 per month to an average of 97 by month 6.

The proportions of babies taking part in both randomisations is important to monitor as this will impact the power of the study. If less than 30% take part in both randomisations, then power will be <80%.

We will also monitor the sample size assumption for missing data which will occur when carers/parents withdraw consent for use of their baby's information in NNRD to be analysed.

The internal pilot will run seamlessly into the main study if all three criteria below are met.

Progression Criteria	Recruitment per comparison	Proportion taking part in both trials	Withdrawn consent for use of NNRD data

RED: Consider Stopping	<322 randomisations in each comparison	<30% (as trial will have <80% power)	>10%
AMBER: Explore approaches to achieve target	322-644 randomisations in each comparison	30-50%	>2% but < 10%
GREEN: Continue without changes	>644 recruited to each comparison	>50%	<= 2%

The Trial Steering Committee will be responsible for assessing the internal pilot outcomes and provide guidance to the trial team.

7.11 Early discontinuation of the study

The study may be prematurely terminated for the following reasons:

- Event/s which in the opinion of the Data Monitoring and Ethics Committee contraindicate/s randomisation of additional participants
- Sponsor decision
- Research ethics committee decision
- Funder decision

An internal pilot will take place for the first 9 months after recruitment starts. It will use traffic light system: if the red criteria are met there will be a discussion with the Trial Steering Committee about stopping the trial.

The Data Monitoring and Ethics Committee Charter will define procedures for early termination of the study due to safety or efficacy, should this be required. As this is a comparative-effective trial embedded in standard care, in the case of early discontinuation, all procedures will continue except the mechanistic sub-study.

8 REGULATORY, ETHICAL AND LEGAL ISSUES

8.1 Declaration of Helsinki

The investigators will ensure that this study is conducted in full conformity with the 2024 revision of the 1964 Declaration of Helsinki.

8.2 Good Clinical Practice

The study will be conducted in accordance with the guidelines laid down by the International Conference on Harmonisation for Good Clinical Practice (ICH GCP E6 guidelines).

8.3 Research Ethics Committee Approval

8.3.1 Initial approval

Prior to the enrolment of participants, the Research Ethics Committee (REC) must provide written approval for the conduct of the study at named sites, the protocol and any amendments, the Participant Information Sheet and Consent Form, any other written information that will be provided

to the participants, any advertisements that will be used and details of any participant compensation.

8.3.2 Approval of amendments

Proposed amendments to the protocol and aforementioned documents will be submitted to the REC for approval. Amendments may be implemented only after a copy of the REC approval letter has been obtained.

Amendments that are intended to eliminate an apparent immediate hazard to participants may be implemented prior to receiving Sponsor or REC approval. However, in this case, approval must be obtained as soon as possible after implementation.

The Trial Management Group will make the decision to amend the protocol and decide whether changes are substantial or non-substantial with support from the Trial Steering Committee, and Data Monitoring and Ethics Committee and advice from the Parent, Patient and Public Involvement and Engagement Advisory Group if applicable.

Changes will be communicated to stakeholders, including participating sites, electronically and version controlled with tracked changes and in accordance with relevant standard operating procedures. The amended protocol will be reviewed by all members of the Protocol Development Group and funder prior to finalising.

8.3.3 End of trial notification

The end of trial notification will be submitted to the REC within 90 days of the end of trial definition being met. In the event of a premature halt of the trial, the timeframe is 15 days, and the reasons should be clearly explained in the notification.

8.4 Health Research Authority approval

Health Research Authority (HRA) approval will be obtained prior to starting the study. Each participating site will confirm capacity and capability prior to commencing.

The HRA and all participating sites also need to be notified of all protocol amendments to assess whether the amendment affects the institutional approval for each site.

8.5 Non-compliance and serious breaches

All protocol deviations and protocol violations will be reported using the eCRF and reviewed by the Chief Investigator and reported to the ICTU Quality Assurance team on a monthly basis. Protocol violations will be reported to the Sponsor.

An assessment of whether the protocol deviation/violation constitutes a serious breach will be made. A serious breach is defined as:

A breach of the conditions and principles of GCP in connection with a trial or the trial protocol, which is likely to affect to a significant degree:

- The safety or physical or mental integrity of the UK trial participants; or
- The overall scientific value of the trial

The Sponsor will be notified within 24 hours of identifying a likely Serious Breach. If a decision is made that the incident constitutes a Serious Breach, this will be reported to the REC within 7 days of becoming aware of the serious breach.

8.6 Insurance, indemnity and sponsor

Imperial College London holds negligent harm and non-negligent harm insurance policies which apply to this study.

Imperial College London will act as the Sponsor for this trial. Delegated responsibilities will be assigned to the NHS trusts taking part in the trial.

8.7 Trial registration

The study will be registered on the ISRCTN registry in accordance with requirements of the International Committee of Medical Journal Editors regulations.

8.8 Contact with General Practitioner

Participation in COLLABORATE will be notified to the General Practitioner in the infant's discharge summary with parent consent in accordance with standard practice for trials in neonatal units.

We will refer incidental findings on MRI that may be clinically actionable to relevant NHS services and inform the child's General Practitioner of their participation in the mechanistic sub-study.

8.9 Participant confidentiality

The investigators will ensure that the participant's confidentiality is maintained. On documents submitted to the Sponsor, participants will only be identified by an ID number. Documents that are not submitted to the Sponsor will be kept in a strictly confidential file by the investigator.

Identifiable data collected for dissemination and long-term follow-up will be stored securely with restricted access.

The investigators shall permit direct access to participants' records and source documents for the purposes of monitoring, auditing, or inspection by the Sponsor, authorised representatives of the Sponsor, NHS, Regulatory Authorities and REC.

8.10 Data protection

The investigators and study site staff will comply with the requirements of the Data Protection Act 2018 concerning the collection, storage, processing, and disclosure of personal information and will uphold the Act's core principles.

8.11 End of trial

The end of trial is defined as when all research data has been captured, and the database is locked for final analysis.

8.12 Study documentation and data storage

The investigators will retain essential documents until notified by the Sponsor and for at least 10 years after study completion. Participant files and other source data (including copies of protocols, eCRF, original reports of test results, correspondence, records of informed consent, and other documents pertaining to the conduct of the study) must be retained. Documents will be stored in

such a way that they can be accessed/data retrieved at a later date. Consideration should be given to security and environmental risks.

No study document will be destroyed without prior written agreement between the Sponsor and the investigator. Should the investigator wish to assign the study records to another party or move them to another location, written agreement must be obtained from the Sponsor.

MRI data will be stored at the University of Edinburgh with guaranteed backup and resilience.

9 DATA MANAGEMENT

9.1 Data Management Plan

A Data Management Plan summarising how data are handled from the first point of data entry through to final analysis, including data querying and cleaning procedures will be prepared during the study set-up phase.

9.2 Source data

Each participating site will maintain appropriate records for this study in compliance with the regulatory and institutional requirements for the protection of confidentiality of participants. Source documents and source data are considered to be the original documentation where participant information, examinations and other information are recorded.

Documentation of source data is necessary for the reconstruction, evaluation and validation of clinical findings, observations and other activities during a study. Each site will permit authorised representatives of the sponsor, its designees, and appropriate regulatory agencies to examine clinical records as required.

9.3 Mechanistic sub-study data

The MRI community has developed methods for anonymising data, namely, ensuring that identifying information (e.g., birth date, sex) is unattached from shared data, and “de-facing” the data. There are several algorithms for defacing MRI data (e.g., FreeSurfer’s mri_deface). We will employ best practices for deidentifying and anonymizing MRI data prior to sharing. We will also ensure that participants understand the way that their data will be shared and obtain their consent. The risk of identification from MRI is low.

Neuroimaging data will be stored on a dedicated GPGPU server, accessible to members of the research team. MRI data acquired in Edinburgh are archived indefinitely in safe, long-term storage in Edinburgh Compute and Data Facility (ECDF). The image processing server is linked by automatic daily back-up to the off-site ECDF. The compute component of ECDF, Eddie, has built-in redundancy with error check and "self-healing,' and is itself backed-up using 2 tape systems.

9.4 National Neonatal Research Database

COLLABORATE is a real-world-data-enabled study. The principal data source, the NNRD, is a HRA approved National Information Asset containing NHS real-world data (43). Data are obtained as a regular, defined extract from the Electronic Patient Record Systems of all NHS neonatal units; these are quality-assured and curated to be “research ready”. Parents are informed about the NNRD and its use for approved research and have opportunity to opt-out of the inclusion of their

baby's data but less than 1% do so. The NNRD is located at Imperial College London and managed by the COLLABORATE Chief Investigator.

A detailed data extraction plan will be developed that defines the data that will be extracted by the NNRD team. Generic names for concomitant medications will be provided by the NNRD team.

9.5 Ancillary data capture

Requirements for data other than those obtained from the NNRD are minimal (consent; randomisation; recruiting site; protocol deviations/violations; SAR; withdrawals; clinical information; maximum alkaline phosphatase, urea, and creatinine during in-patient stay as indices of metabolic bone disease, protein intake, and renal function, respectively) and will be obtained through Electronic Data Capture (EDC) using a dedicated eCRF system (OpenClinica).

Data will be entered into the EDC system by trained site personnel. All data recorded in the eCRF will be signed off by the Investigator or his/her appropriate designee. All changes made following initial submission of data will have an electronic audit trail with a date. Details of procedures for eCRF completion will be provided in a study manual.

9.6 Language

9.6.1 eCRF

The eCRF will be in English.

9.6.2 Parent information

Parent information will be available in English, Welsh and the ten other most prevalent UK languages (Polish; Romanian; Punjabi; Urdu; Portuguese; Spanish; Arabic; Bengali; Gujarati; Italian.

9.7 Archiving

All documentation, including that held at participating sites and the study coordinating centre, will be archived for a minimum of 10 years following the end of the study. The University of Edinburgh provides a DataVault data retention service to archive the MRI research data. A master copy of new data will be migrated onto this service as part of the process of data curation at the end of the study. Pseudonymised data will be stored in DataVault for 10 years; at the end of that period the data will be anonymised.

10 STUDY MANAGEMENT STRUCTURE

10.1 Trial Management Group

A Trial Management Group (TMG) will be convened including the Chief Investigators, Co-Investigators, key collaborators, Trial Statistician, Trial Manager, and Parent, Patient and Public Involvement and Engagement (PPPIE) lead. The TMG will be responsible for day-to-day conduct of the trial and operational issues. Details of membership, responsibilities and frequency of meetings will be defined in separate Terms of Reference.

10.2 Trial Steering Committee

A Trial Steering Committee (TSC) will be convened including as a minimum an independent Chair, independent clinician, independent statistician, the Chief Investigators, the Trial Manager, and two PPPIE representatives. The role of the TSC is to provide overall supervision of trial conduct and progress. Details of membership, responsibilities and frequency of meetings will be defined in a separate charter.

10.3 Data Monitoring and Ethics Committee

A Data Monitoring and Ethics Committee (DMEC) will operate in accordance with the Damocles Group charter (44) and comprise 4-6 members including PPPIE members and a Chair with relevant previous experience, to facilitate effective interactions, and good understanding of both clinical and statistical issues. The DMEC will receive reports supplied in confidence and will advise the TSC on whether the accumulated data from the study, together with results from other relevant research, justifies continuing recruitment. The DMEC will meet first during trial set-up to agree the content of the charter, at the end of the pilot phase and then at 50% and 75% recruitment. Details of membership and responsibilities will be defined in a separate charter.

10.4 Parent, Patient and Public Involvement and Engagement (PPPIE) Advisory Group

This will be an informal group led by the study PPPIE lead. Membership will not be fixed and will include representatives from charity co-investigators Bliss (the national preterm and sick newborn charity) and the Adult Preemie Advocacy Network as well as from collaborating organisation NEC UK, alongside other community partners and PPPIE representatives. Their role will be to offer informal advice to the TMG and study investigators on relevant issues arising during the study and in relation to dissemination of outcomes.

10.5 Scientific Advisory Group

This will be informal group chaired by the CI. Membership will not be fixed. Their remit will be to provide scientific advice to the to the TMG and study investigators on relevant issues arising during the study, and dissemination of outcomes, and additionally to relevant external researchers planning to develop mechanistic protocols for COLLABORATE bolt-on studies, and/or related global studies.

10.6 Young Investigator Group

This informal group will be chaired by Co-I Dr Uthaya. Their remit will be to advise external researchers and Clinical Trial Units who wish to utilise the NNRD for future studies.

11 RISK ASSESSMENT AND MONITORING

11.1 Study-specific risk assessment

As this is a comparative-effective trial investigating treatments that are already in widespread, routine use, this study is classified ‘low’ risk.

11.2 Monitoring

The study will be monitored periodically by ICTU staff to assess progress, verify adherence to the protocol, ICH GCP E6 guidelines and other national requirements and review the completeness, accuracy and consistency of the data. Monitoring procedures and requirements will be documented in a Monitoring Plan.

11.3 Quality control and quality assurance

Quality control will be performed according to ICTU internal procedures. The study may be audited by a Quality Assurance representative of the Sponsor and/or ICTU.

The study may be subject to inspection and audit by regulatory bodies to ensure adherence to GCP and the NHS Research Governance Framework for Health and Social Care (2nd Edition).

11.4 Peer review

This study has been awarded funding following independent peer review by the National Institute of Health Research.

12 PARENT, PATIENT, PUBLIC INVOLVEMENT AND ENGAGEMENT

12.1 Study delivery

The study PPPIE lead, will ensure continuing involvement supported by co-investigators Bliss (the national preterm and sick baby charity), APAN (Adult Preemie Advocacy Network), collaborating organisation NEC UK (national charity), and community partners, people with experience of neonatal care that have strong links with their communities. We will recruit a PPPIE advisory group of 6-8 people to offer advice as the study is rolled out and to assist with dissemination of findings (see section 10.4 above). All trial committees will include PPPIE representatives.

12.2 Study development

PPPIE has played an integral part in the development of this proposal. Our work has shown that parents and former neonatal patients have a good understanding of the uncertainties we aim to resolve. Their contributions helped develop the study in several ways (45-47).

12.2.1 Information provision by clinical teams

Mothers relayed the pressure they experienced to express breast milk. Focus groups identified the importance of using language that does not add to guilt when unable to provide enough. Some parents expressed distaste at the thought of donor milk; others worried about formula.

Focus groups also elicited the novel finding that many parents, especially those with experience of NEC, felt the opportunity to participate in a randomised trial to resolve feeding uncertainties, would relieve anxieties brought about by not knowing which care practice was best. Parents expressed strong altruism in wanting to benefit other babies. We will convey these insights to recruiting neonatal teams to ensure that discussions about expressing breast milk do not increase parent distress and that they are aware that rather than shying away from participation, parents want their babies and other infants, to benefit through research.

12.2.2 Digital PARCA-R

We involved parents in the development of a digital version of the PARCA-R, a validated questionnaire to assess language and cognitive development at age 2-years (24). Parents helped

evaluate ease of use, acceptability, and consent for data sharing. The digital PARCA-R will be made available to neonatal units as part of this study.

12.2.3 Parent Information Sheet

Parents told us information leaflets should not be “dumbed down” versions of scientific information but written honestly, with the full diversity of parents in mind. They helped us co-create a Parent Information Leaflet.

12.2.4 Video clips

Parents suggested we provide short video clips voiced by parents that explain the study.

13 PUBLICATION AND DISSEMINATION POLICY

13.1 Disclosure

Information concerning the study, patent applications, processes, scientific data or other pertinent information is confidential and remains the property of the Sponsor. The investigator may use this information only for the purposes of the study.

It is understood by the investigator that the Sponsor will use information developed in this clinical study and, therefore, may disclose this as required to other clinical investigators.

Verbal or written discussion of results prior to study completion and full reporting should only be undertaken with written consent from the Sponsor.

13.2 Study results

Results will be published or presented by the investigators, but the Funder will be given the opportunity to review and comment on planned publications prior to submission in accordance with their requirements. The investigators understand that they have an obligation to provide complete study results, and all data developed during this study to the Sponsor. Permission from the Chief and all co-investigators is necessary prior to disclosing any information relating to this study outside of the TSC and DMEC. Any request by site investigators or other collaborators to access the study dataset must be formally reviewed by the TSC.

13.3 Authorship of publications

Authorship of all publications will follow International Committee of Medical Journal Editors guidelines.

13.4 Final report

A final report summarising the study results will be submitted to the REC/HRA within a year of the end of study.

13.5 Notification of study results to parents/guardians

Participant’s parents/legal guardians will be notified of study results at two time points; first upon publication of outcomes to neonatal unit discharge, and second, upon publication of age two-year outcomes.

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15 REVISION HISTORY

Version	Date	Summary of changes
1.0	21/08/2025	First version
2.0	22/10/2025	Changes following initial Ethics Committee review. • Clarification of end of trial definition • Addition of Trial Manager name • Addition of REC reference number
3.0	09/01/2026	• Addition of ISRCTN number • Minor updates to nutritional supplements and faltering growth section and clarification that further guidance will be provided in the study manual • Addition of study statistician name • Formatting, typographical and grammatical updates • Updated Schedule of Assessments table for further clarity • Minor clarifications to section 5.2.2.2 (For babies participating in the mechanistic sub-study), 5.8 (Lab evaluations), and 6.8 (Adverse reaction reporting) • Update to MRI window for mechanistic sub-study to 37-44 weeks PMA

SIGNATURE PAGE 1 (CHIEF INVESTIGATOR)

The signature below constitutes approval of this protocol by the signatory, on behalf of the Protocol Development Group, and provides the necessary assurances that this study will be conducted according to all stipulations of the protocol including all statements regarding confidentiality.

Study Title: COLLABORATE: An efficient, UK-wide, real-world-data-enabled, group-sequential, randomised controlled trial to determine clinical efficacy, effect size, and safety of widely used enteral feeds in reducing necrotising enterocolitis, mortality, and cognitive impairment in extremely preterm babies

EDGE Number: 181495

Signed: _____

Neena Modi
Professor of Neonatal Medicine

Date: _____

SIGNATURE PAGE 2 (SPONSOR)

The signatures below constitute approval of this protocol by the signatory.

Study Title: COLLABORATE: An efficient, UK-wide, real-world-data-enabled, group-sequential, randomised controlled trial to determine clinical efficacy, effect size, and safety of widely used enteral feeds in reducing necrotising enterocolitis, mortality, and cognitive impairment in extremely preterm babies

EDGE Number: 181495

Signed: _____

Ruth Nicholson
Head of Research Governance and Integrity
Imperial College London

Date: _____

SIGNATURE PAGE 3 (STATISTICIAN)

The signatures below constitute approval of this protocol by the signatory.

Study Title: COLLABORATE: An efficient, UK-wide, real-world-data-enabled, group-sequential, randomised controlled trial to determine clinical efficacy, effect size, and safety of widely used enteral feeds in reducing necrotising enterocolitis, mortality, and cognitive impairment in extremely preterm babies

EDGE Number: 181495

Signed: _____

Victoria Cornelius
Professor of Medical Statistics and Trials Methodology
Imperial College London

Date: _____

SIGNATURE PAGE 4 (PRINCIPAL INVESTIGATOR)

The signature of the below constitutes agreement of this protocol by the signatory and provides the necessary assurance that this study will be conducted at his/her investigational site according to all stipulations of the protocol including all statements regarding confidentiality.

Study Title: COLLABORATE: An efficient, UK-wide, real-world-data-enabled, group-sequential, randomised controlled trial to determine clinical efficacy, effect size, and safety of widely used enteral feeds in reducing necrotising enterocolitis, mortality, and cognitive impairment in extremely preterm babies

EDGE Number: 181495

Address of Institution: _____

_____

_____

Signed: _____

Print Name and Title: _____

Date: _____

APPENDICES

APPENDIX 1: STUDY FLOW CHART