BMJ Open

Recombinant Surfactant protein D to prevent neonatal chronic lung disease (RESPONSE): a protocol for a phase I safety trial.

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2024-086394
Article Type:	Protocol
Date Submitted by the Author:	14-Mar-2024
Complete List of Authors:	Bhatt, Reena; University College London Hospital Neonatal Intensive Care Unit; University College London, Neonatology Madsen, Jens; University College London, Department of Neonatology Castillo-Hernandez, Tania; University College London, Department of Neonatology Chant, Kathy; University College London, Department of Neonatology Dehbi, Hakim-Moulay; University College London Institute of Clinical Trials and Methodology Marlow, Neil; UCL Clark, Howard; University College London Hospitals; University College London, Department of Neonatology
Keywords:	IMMUNOLOGY, Neonatal intensive & critical care < INTENSIVE & CRITICAL CARE, RESPIRATORY MEDICINE (see Thoracic Medicine)

Title

Recombinant Surfactant protein D to prevent neonatal chronic lung disease (RESPONSE): a protocol for a phase I safety trial.

Names protocol contributors

Reena Bhatt[1,2], Jens Madsen[2], Tania Castillo-Hernandez[2], Kathy Chant[2], Hakim-Moulay Dehbi[3], Neil Marlow[2], Howard Clark[1,2]

[1] Neonatal Intensive Care Unit, EGA Wing, University College London Hospitals, London, United Kingdom

[2] Department of Neonatology, EGA Institute for Women's Health, University College London, London, United Kingdom

[3] Comprehensive Clinical Trials Unit, University College London, London, United Kingdom

Corresponding author

Dr Reena Bhatt

EGA Institute for Women's Health

Rockefeller Building

University College London

21 University Street

London, WC1E 6DE

United Kingdom

Tel: 02076796031

Email: reena.bhatt@ucl.ac.uk

Abstract

Introduction: Chronic respiratory morbidity from bronchopulmonary dysplasia (BPD) remains the most common complication of preterm birth and has consequences for later respiratory, cardiovascular and neurodevelopmental outcomes. The early phases of respiratory illness are characterised by rapid consumption of endogenous surfactant and slow replenishment. Exogenous surfactant is routinely administered to infants born before 28 weeks of gestation as prophylaxis.

Endogenous surfactant includes four proteins, known as surfactant proteins (SP) A, B, C and D. Current bovine- and porcine-derived surfactant preparations only contain surfactant proteins B and C. SP-D has a key role in lung immune homeostasis as part of the innate immune system. Laboratory studies using recombinant SP-D have demonstrated reduced inflammation, which may be a pathway to reducing the associated morbidity from BPD. RESPONSE utilises a recombinant fragment of human surfactant protein D (rfhSP-D), in a phase I safety and dose-escalation trial as the first stage in determining its effect in humans.

Methods and Analysis: This is a single centre, dose-escalation, phase I safety study aiming to recruit 24 infants born before 28 weeks gestation with respiratory distress syndrome (RDS). In addition to routine surfactant replacement therapy, participants will receive three doses of rfhSP-D via endotracheal route at either 1mg/kg, 2mg/kg or 4mg/kg. The study utilises a Bayesian Continual Reassessment Method (CRM) to make dose escalation decisions. Dose-limiting events (DLE) in this trial will be graded according to the published neonatal adverse event severity score (NAESS). The primary outcome of this study is to evaluate the safety profile of rfhSP-D across each dose level based on the profile of DLE to establish the recommended phase 2 dose (RP2D) of rfhSP-D.

Ethics and Dissemination: The RESPONSE study has received ethical approval. Results from the study will be published in peer-reviewed journals and presented at national and international conferences.

Trial registration: Medical EudraCT: 2021-001824-16, ISRCTN: 17083028, Clinical trials.gov.uk: NCT05898633.

Protocol Version: RESPONSE Protocol v3.0 25/01/2024

Funding Statement: This work is supported by the Medical Research Council (MRC) DPFS grant, grant number MR/P026907/1.

Name and contact information for the trial sponsor: University College London (UCL) with sponsor responsibilities delegated to the Comprehensive Clinical Trails Unit (CCTU). Contact: cctu.response@ucl.ac.uk

Role of sponsor: Specific functions have been delegated to the UCL CCTU by the sponsor. A clinical project manager at the UCL CCTU will oversee the clinical trial manager who will be responsible for the day-to-day management of the trial. The CCTU staff will be involved in site initiation, database construction, development of the protocol and trial-related documentation. The sponsor will be responsible for the audit of the trial.

Key Words: Neonates, bronchopulmonary dysplasia, surfactant protein D, prematurity, Bayesian method

Article Summary:

Strengths:

- This study uses the International Neonatal Consortium (INC) Neonatal Adverse Event Severity Score (NAESS) which is specific to this population and allows a better grading and understanding of the adverse events and progression of the trial. This scoring system unlike others e.g. Common Terminology Criteria for Adverse events (CTCAE) takes into account age-appropriate behaviour e.g. feeding and physiological parameters such as changes in oxygenation. Although the NAESS has not been rigorously validated, it is well-placed to improve the quality of drug evaluation in this highly vulnerable population.
- This is a safety study aiming to establish a recommended phase II dose of a novel therapy in a highly vulnerable population affected by Bronchopulmonary dysplasia which has a significant impact on long-term lung health.
- This study utilises Bayesian analysis which utilises prior cohort data to inform the ongoing dose escalation.

Limitations:

- This is a single-centre study which may affect recruitment and the population characteristics.

Introduction

Clinical Need for Study

The introduction of exogenous surfactant replacement therapy has significantly improved mortality in extremely preterm infants, those born before 28 weeks of gestation (1). Despite this, chronic respiratory morbidity from BPD remains the most common complication of very preterm birth. BPD may be formally defined by the persisting need for respiratory support past 36 weeks postmenstrual age (PMA) (2). It affects up to 75% of extremely preterm infants (3), with decreasing prevalence with increasing gestational age (4). The pathogenesis of BPD is complex and multifactorial, involving lung immaturity, infection, inflammation, oxygen toxicity and ventilator-induced injury.

Unlike when first described, BPD is now rarely seen in infants born at more than 1200 g or after 30 weeks of gestation (2, 5, 6) due to the introduction of antenatal steroid administration, surfactant replacement therapy, improved ventilation strategies and better nutrition (7, 8). The prevalence of BPD has not fallen as expected (8-10). It can be argued that with advances in neonatal care leading to increased survival of infants at greatest risk of BPD, the prevalence may increase in years to come presenting a challenge for healthcare systems worldwide. Furthermore, BPD has lifelong consequences, with respiratory impairment that has important implications for adult clinicians, tracking through to adult life (11, 12) and neonatal BPD is also a marker for adult cognitive, educational and behavioural impairment with implications for health, wealth and relationships for life (13).

As the mean gestational age of neonatal populations has fallen with increasing survival, the pathophysiology of chronic respiratory disease in very preterm populations has changed. Whereas the original descriptions of BPD related the occurrence and progression primarily to barotrauma from mechanical positive pressure ventilation (14), with increasing immaturity the profile of causation has changed, and this “new” BPD (15) is primarily found among extremely preterm infants. The primary driver in its development is lung inflammation, subject to the other risks referred to above. The disease is characterised by developmental arrest of lung tissue and a loss of alveolar septation by impairing alveolar crest development. This

interruption in normal lung development with superimposed inflammation, oxygen toxicity and pressure-induced changes (barotrauma, volutrauma, atelectotrauma) completes the clinical picture.

Postnatal pulmonary inflammation is due to an imbalance in humoral factors favouring a pro-inflammatory response (16, 17) and increased presence of inflammatory cells in the airway (18). Inflammation, secondary to positive pressure ventilation, oxygen therapy or infection, may have further impact on the cytokine profile and the interruption of lung development. The overwhelming evidence for inflammation as a causal mechanism in the development of BPD suggests that early anti-inflammatory therapies might reduce the frequency and severity of the condition. Identification of potential therapeutic targets remains a goal to reduce the frequency of BPD in high-risk infants. Naturally occurring SP-D has gained increasing interest as a potential immunotherapy to dampen the pro-inflammatory cascade and facilitate lung repair, thus reducing the frequency and severity of lung disease. In turn, this may have important long-term benefits for the child.

Surfactant Protein D

Mammalian surfactant comprises largely phospholipids (80%), neutral lipids (10%) and surfactant proteins (10%), dipalmitoylphosphatidylcholine (DPPC) being the primary surface-active component at the alveolar surface (19). Four SP are found in surfactant, SP-A, SP-B, SP-C and SP-D. SP-B and SP-C are hydrophobic and their role is largely to stabilise the lipid monolayer formed at the air-liquid interface by stimulating phospholipid adsorption and reducing surface tension. Due to their hydrophobic nature, these SP are easily extracted from bovine or porcine sources and present in widely used commercial surfactants. In contrast, SP-A and SP-D are hydrophilic and are not present in the surfactant preparations currently used in clinical practice.

SP-D is an essential lung component and functions to keep the lungs in a hypo-responsive state at rest, free from aberrant inflammation and infection. The actions of SP-D include aggregation of pathogens, antimicrobial activity against pathogens such as *Klebsiella,* increased phagocytosis and clearance of apoptotic cells, and regulation of mediator production (20). SP-D consists of four main regions which include an N-terminal domain, a collagenous tail, a neck region and a carbohydrate recognition domain; it exists as a

trimer. Through its carbohydrate recognition domain, SP-D binds carbohydrates in a calcium-dependent manner (20, 21) and via the N-terminal region, the trimeric units oligomerise to give rise to a dodecameric cross-like structure. These can further form oligomers or 'stellate multimers', which increases the strength to bind carbohydrates and agglutinate various pathogens (20).

SP-D levels in preterm infants and evidence for recombinant fragment Surfactant protein D as a therapeutic agent

Bronchoalveolar lavage (BAL) samples taken from preterm infants over the first few days after birth have demonstrated low concentrations of SP-D in association with RDS that were associated with an increased risk of BPD (22, 23). Binding assay studies evaluating the lectin activity of SP-D demonstrate that the SP-D present in the BAL of preterm infants was less effective than that in term infants (23). Sepsis in preterm infants can be life-threatening and contributes significantly to the inflammation seen in BPD. Further, SP-D concentrations increase in preterm infants in the presence of sepsis, demonstrating its potential role as an acute phase reactant (24). Given the known interactions of SP-D to bacterial, viral and fungal pathogens (20, 25), intervention with SP-D would be expected to promote their clearance in this vulnerable population and reduce further damage. Finally, in SP-D knock-out mouse models (26), emphysematous changes are seen that are similar to those seen in the lungs of preterm infants.

Given these homeostatic and anti-inflammatory roles of SP-D, it is an attractive target for therapy, and if administered early to preterm infants there would be a reduction in inflammation by down-regulation of the pro-inflammatory signalling pathways in addition to interaction with common pathogens that induce inflammation such as *Escherichia coli. In vivo* studies using preterm lambs given recombinant full-length SP-D in addition to commercially available surfactant (which lacks SP-A and SP-D) showed a clear reduction in the pro-inflammatory cytokines such as interleukin-8 (IL-8) (27), which provides encouraging data for its potential clinical use in this population.

In practice, the properties of full-length SP-D (including varying degrees of oligomerisation, limited solubilisation and potential aggregation at higher concentrations) make it difficult to develop a stable

preparation that could be administered. Therefore, recombinant fragments of human SP-D have been explored in translational models as a potential therapy for BPD. Pre-clinical data showed the efficacy of rfhSP-D treatment in reducing and correcting inflammation in chronic inflammatory lung disease caused by SP-D deficiency. SP-D knock-out mice develop symptoms of chronic obstructive pulmonary disease (COPD) and emphysema relevant to BPD, which are correctable following treatment with recombinant SP-D (26, 28).

A stable form of rfhSP-D has been produced using a mammalian cell line and purified using affinity chromatography using a *N*-Acetylmannosamine (ManNAc)-coupled matrix as described previously (29). The recombinant fragment comprises the neck, CRD and eight gly-Xaa-Yaa repeats similar to that described for a bacterially expressed recombinant fragment of human SP-D (30). The carbohydrate recognition domain is the functional anti-inflammatory and anti-infective part of the protein without the long collagenous tail and the suggested pro-inflammatory N-terminal region (30). The rfhSP-D proposed as an investigational medicinal product (IMP) retains its anti-inflammatory properties when used as an adjunct to exogenous surfactant therapy administered via an endotracheal tube in a well-established translational model using preterm ventilated lambs (31). The endotoxin content is less than 0.05 EU/mg rfhSP-D.

Justification for the dosage regimen in the safety trial

The proposed regimen is based on the estimation of human equivalent dosages based on effective dosing in animals. In murine studies, the replacement dose of rfhSP-D was 10 micrograms daily. Assuming an average mouse weight of approximately 10-20 g, this approximates to 1 to 2 mg/kg per day. The effective dose of rfhSP-D in the preterm lamb has been estimated to be 1.5 mg/kg (unpublished data). In current practice, the administration of 100-200 mg/kg of surfactant replacement would contain 1-4 mg/kg if a naturally occurring product was used. Hence after due consideration, we elected to trial three potential doses of rfhSP-D, namely 1, 2 and 4 mg/kg.

Study Objectives

RESPONSE is a phase I study and aims to assess the safety of 3 intratracheal doses (1 mg/kg, 2 mg/kg and 4 mg/kg) of rfhSP-D in extremely preterm ventilated infants at risk of BPD.

The primary objectives are:

- To assess the safety profile of rfhSP-D across 3 dose levels based on the occurrence of dose-limiting events (DLEs) as defined below.
- To establish the Recommended Phase 2 Dose (RP2D) of rfhSP-D for preterm infants born before 28 weeks of gestation.

Secondary objectives are:

- To evaluate systemic absorption of rfhSP-D using serial measurements of SP-D in plasma and its continued presence in tracheal fluid.
- To determine the effect of rfhSP-D on inflammatory markers in the lung secretions (e.g. cell counts of neutrophils, macrophages, IL-8, IL-6, IL- 1).
- To compare the clinical effects of intratracheal administration of rfhSP-D on physiological and intensive care parameters in treated infants in this trial with non-treated infants from a parallel observational cohort study of untreated infants.

Methods and Analysis

Trial design

The study will be conducted in a single-centre, tertiary level 3 neonatal intensive care unit. This study utilises a Bayesian continual reassessment model (32-34), a model-based design that informs how the dosage of rfhSP-D should be adapted for the next participant cohort based on past trial data. For this first-in-human study, a dose escalation design will be used (Figure 1).

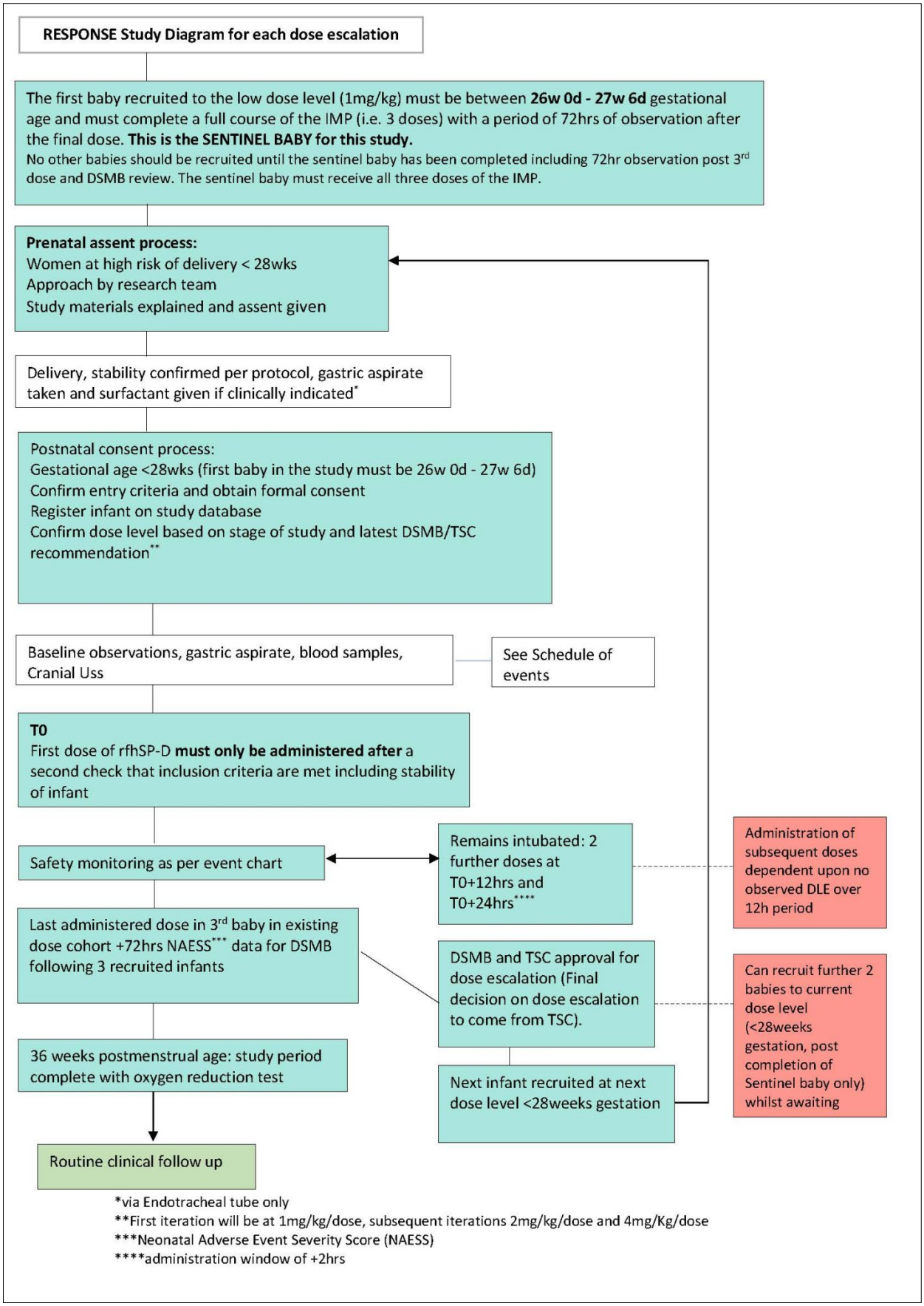


Figure 1. Dose escalation process in RESPONSE using rfhSP-D in preterm infants at risk of BPD.

The three dose levels to be considered are 1mg/kg/dose, 2mg/kg/dose and 4mg/kg/dose. Participants will be enrolled at each dose level with a minimum of three participants per dose level. Each participant will receive 3 doses of rfhSP-D at 0 hours, 12 hours and 24 hours provided that they continue to meet the inclusion criteria and are clinically stable. The first dose of rfhSP-D will be administered within two hours of standard surfactant therapy being given. Whether or not the dose level is escalated will depend on the occurrence of DLEs in all current participants and the doses they have received. A model will be used to estimate the risk of DLE per dose level. Initial estimates of these risks will be updated using data collected throughout the trial. A one-parameter empiric model will be used to describe the relationship between the dose and the probability of observing a DLE. The CRM model will not allow dose-skipping. The target level of dose-limiting events level is set at no greater than 20%. Before the trial, the parameter of the model will be assigned a non-informative prior distribution and initial estimates of DLE probabilities will be derived using model calibration. The recommended phase two dose will be defined by considering safety and will be the highest dose level that has an estimated probability of DLE closest but below the target DLE level of no greater than 20%.

Dose escalation procedure

A schema of the dose escalation procedure and review is shown in Figure 2. The sentinel baby is the very first baby recruited to the study and this baby must be greater than 26 weeks gestational age (GA). The sentinel baby must have received all three administrations of the investigational medicinal product (IMP) and have had 72 hours of observed data post administration of 3rd administration before further infants can be recruited for the study. If the first participant does not receive all three doses of the IMP then data will still be collected but they will not qualify as the sentinel baby for this study. All infants recruited after the sentinel baby will be from 23 weeks to 27 weeks and 6 days GA for the remainder of the study.

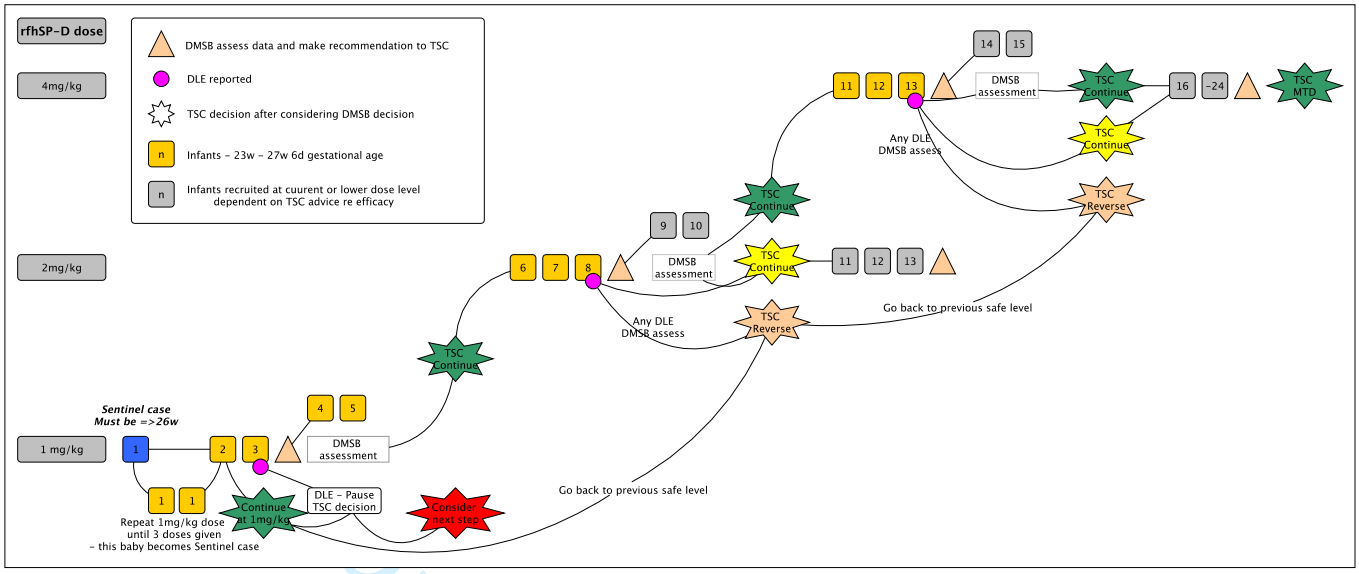


Figure 2. Schematic overview of the dose escalation design using the Continual Reassessment Method. This schematic overview illustrates the decisional pathway planned. The Data and Safety Monitoring Board (DSMB) will review the clinical outcomes at the points indicated and make recommendations to the Trial Steering Committee (TSC), who in turn will report their decision to the Trial Management Group to action. Potential actions are to continue to recruit/escalate dose/return to previous safe dose or to pause/stop the trial. Whilst the DSMB and TSC are deliberating, further infants may be recruited to the lowest or the previous dose (if escalation has occurred). The MTD is the maximum tolerated dose in the absence of DLEs. The DSMB may recommend to the TSC that recruitment to lower (i.e. safe) doses occurs to achieve even recruitment numbers to facilitate later evaluation for efficacy.

Continuous recruitment model during dose escalation decision period

The rationale for continuous recruitment in this trial is to minimise delays to recruitment during the DSMB review which takes place after a minimum of 3 participants at a dose level. It also allows for the trial of the IMP in a larger number of participants at the lower dose levels, allowing for better characterisation of the dose-response curve and the safety profile of rfhSP-D. This means that in the 1 mg/kg and 2 mg/kg cohorts, up to a further 2 participants can be recruited whilst the DSMB review dose escalation provided that no DLE has occurred in the first three participants of the dose cohort. The continued recruitment of up to two additional participants at the same or lower dose level, whilst the DSMB conduct their review, will only be permitted if there are no concerns that a DLE has occurred in the cohort under review i.e. the first 3 infants under review. Any adverse event data collected for the additional two recruited participants during DSMB review will then be reviewed by the DSMB once the 72 hour follow-up period is completed. If at any point there are concerns regarding DLEs in these additional participants, but dose escalation has occurred, then this may lead to a de-escalation. The data from the additional participants will be included at that

point in the CRM which may recommend dose de-escalation in the middle of the next cohort until further data can be reviewed by the DSMB and the TSC at the next opportunity. This methodology of continual reassessment ensures that infants are only treated at the safest dose level whilst the safety profile is characterised.

Study intervention and outcomes

Eligibility criteria

All preterm infants born before 28 weeks of gestation, intubated and treated with surfactant for RDS who are considered clinically stable are eligible. Eligibility will be confirmed within 2 hours of admission to the neonatal unit and re-confirmed for each participant before the IMP is administered.

Inclusion criteria

- Inborn infants born at between 23 weeks and 0 days and 27 weeks and 6 days of gestation (<28 weeks), who are:
    - Intubated or intubation planned for RDS at the time of eligibility check within 12 hours from the time of birth.
    - Receiving standard surfactant replacement therapy.
    - Clinically stable on mechanical ventilation - clinical stability is defined at the time of IMP instillation and is defined below.
- Written informed consent from parents/guardians/person with legal responsibility has been given.

Definition of clinical stability:

Eligibility of the participant must be rechecked prior to administration of the IMP given the varying clinical status of these infants. Stability will consider if the following are true:

- Blood gas parameters within the normal range for preterm infants (pH>7.20; $paCO_2$ <8kPa).
- Mean blood pressure with or without inotropic support at a value in mmHg at least numerically equivalent gestational age in weeks or above.

- No evidence of a pneumothorax.
- Clinical observations within acceptable range for an infant of that gestational age.
- The attending neonatologist considers the infant to be clinically stable.

Exclusion Criteria

- Congenital anomalies (i.e. any major antenatal diagnosed congenital abnormality) such as congenital heart disease, suspected or known chromosomal abnormalities.
- Infants requiring only non-invasive respiratory support i.e. no endotracheal intubation
- Infants born in very poor condition and judged too sick or unstable to be included (high risk of imminent mortality) in an experimental first-in-human study; for example, infants that require maximal intensive care therapy and have findings such as a grade IV intraventricular haemorrhage that may be life-limiting.
- Infants that are born outside the participating site.
- Participation in any other interventional study (participation in another observational study is permissible).
- Parents/legal guardians are unable to give consent due to learning or other difficulties.

Recruitment and Informed consent.

The study team will monitor admissions of any women in threatened or established preterm labour. Any identified women will be approached by the study team to discuss and consider the study and verbal consent for participation will be taken. All parents/legal guardians of eligible participants will be approached if the baby is born and remains eligible for informed written consent. If the person(s) providing consent on behalf of the infant does not speak English, every effort will be made to use translational service to provide an opportunity to participate in the study. If the investigator is not able to confidently take informed consent the infant will not be recruited to the study.

Study Intervention

The recombinant fragment of surfactant protein D drug product has been manufactured to good manufacturing practice (GMP) and is known as rfhSP-D in this study. The sterile IMP is formulated in 0.9% saline at a concentration of 1 mg/mL in 2mL vials. The first administration of rfhSP-D will be given as soon as

possible (within 2 hours) after administration of standard-of-care surfactant therapy, this will be known as T0. Subsequent administration will be given at T0+12 hours and T0+24 hours. If the infant requires further standard surfactant therapy which coincides with the time of IMP administration, then the IMP should be given after the standard surfactant therapy has been administered. The IMP will be administered via a surfactant giving set that is inserted into the endotracheal tube. If the participant is extubated before any IMP dose is scheduled, then the IMP will not be administered. Vital signs will be monitored every 15 minutes for the first hour after administration of the IMP. The study drug can be administered by any authorised medically trained delegate. Eligibility criteria will be confirmed prior to each administration.

Criteria for discontinuing participation in the trial.

Any dose modifications in this protocol will be in line with the trial design and according to the dose level confirmed by the DSMB and the TSC.

Reasons that the intervention may be discontinued are:

- Any change in the infant's condition that in the clinician's opinion justifies the discontinuation of treatment.
- Withdrawal of consent for treatment by the parent/guardian/legal representative.

Participants who discontinue protocol treatment, for any of the above reasons, will remain in the trial for follow-up and data analysis. The study team do not anticipate problems with intervention adherence given that RESPONSE is an inpatient-based study. All participants in the study will receive standard neonatal care and there will be no alteration in their clinical management. The study does not require any additional follow-up of the participants recruited. The hospital where the participant is being cared for is responsible for any medical care. The sponsor holds indemnity for any trial-related harm caused to the participant.

Study Outcomes

The primary outcome of this study is to assess the safety profile of rfhSP-D across three dose levels and to identify the RP2D. DLEs will be identified using clinical criteria and grading as described below.

Dose Limiting events (DLE)

The severity of all adverse events (AE) and/or adverse reactions (AR)s (serious and non-serious) in this trial will be graded using the toxicity graded in the NAESS v1.0 (35). The NAESS has been developed as existing scores such as the Common Terminology Criteria for Adverse events (CTCAE) is not suitable for use in a study involving neonates. The NAESS developed by the International Neonatal Consortium has been developed to facilitate the conduct and appropriate interpretation of neonatal clinical trials such as RESPONSE (36).

Grades for neonatal-specific adverse events according to the NAESS v1.0 (35) are:

Grade 1: Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; no change in baseline age-appropriate behaviours*; no change in baseline care or monitoring indicated.

Grade 2: Moderate; resulting in minor changes of baseline age-appropriate behaviour*; requiring minor changes on baseline care or monitoring*+.

Grade 3: Severe; resulting in major changes of baseline age-appropriate behaviour* or non-life-threatening changes in basal physiological processes+ requiring major change in baseline care or monitoring**

Grade 4: Life-threatening; resulting in life-threatening changes in basal physiological processes+; requiring urgent major change in baseline care***.

Grade 5: Death

*Age-appropriate behaviour refers to oral feeding, voluntary movements and activity, crying pattern, social interactions and perception of pain .
+ Basal physiological processes refers to oxygenation, ventilation, tissue perfusion, metabolic stability and organ functioning
** Minor care changes constitute: brief, local, non-invasive or symptomatic treatments
*** Major care change constitute: surgery, addition of long-term treatment, and upscaling care level.

The DSMB will determine the occurrence of a DLE based on the following criteria:

- A single event defined as Grade 3 or above on the NAESS that is possibly, probably or definitely thought to be related to the IMP. Relatedness will be confirmed by an independent neonatologist at the participating site.
- A single serious adverse event (SAE) that is possibly, probably, or definitely thought to be related to the IMP. Relatedness will be confirmed by an independent neonatologist at the participating site.
- If multiple events meet the criteria for grade 2 on the NAESS, and there are concerns that these may

be related to the IMP. These events will be considered medically important events and will be reported using the same SAE reporting process.

Secondary outcomes related to efficacy for this study are:

- Evaluation of systemic absorption of rfhSP-D using serial measurements of SP-D in plasma and its continued presence in tracheal fluid.
- To determine the effect of rfhSP-D on inflammatory markers in the lung secretions (e.g. cell counts of neutrophils, macrophages, matrix metalloproteinases, neutrophil elastase, IL-8,IL-6, IL-1).
- To compare the clinical effects of endotracheal administration of rfhSP-D on physiological and intensive care parameters in treated infants in this trial with non-treated infants from a parallel observational study.

Participant timeline

The first administration of rfhSP-D will be given as soon as possible (within 2 hours) after administration of standard-of-care surfactant therapy, this will be known as T0. Subsequent administration will be given at T0+12 hours and T0+24 hours. Eligibility and screening investigations will be done before each administration of the IMP as shown in the schedule of events table 1.

Study Visit	Screening	Baseline	Pre Instillation of IMP T0	Pre Instillation of IMP T0 +12h	Pre Instillation of IMP T0 +24h
Time Windows	-	-	<15mins prior to instillation	<15mins prior to instillation	<15mins prior to instillation
Informed consent	*				
Eligibility	*				
Clinical stability		*	*	*	*
Demographics (incl. Gestational Age)	*	*			
Pregnancy and delivery history		*			
Stabilisation history		*			
Clinical assessment (anomalies)	*				
Vital signs	*	*		*	*
Oxygen concentration	*	*		*	*
Ventilator modality		*		*	*
Ventilator settings		*		*	*
Haematology (as per Standard of Care)		*			*
Biochemistry (as per Standard of Care)		*			*
Cytokine levels (plasma)		*			*
Cell counts GA/ETA		*		*	*
Surfactant replacement	*				
Plasma SP-D and rfhSP-D levels		*		*	*
Blood gases	*	*		*	*
SP-D levels GA		*			
rfhSP-D and SP-D level ETA				*	*
Concomitant medication		*		*	*
Cranial ultrasound scan		*			*
Review of AEs and SAEs (from time of consent)		*	*	*	*
Review of DLEs		*	*	*	*

Table 1. Schedule of events at screening and prior to administration of the IMP

Further clinical data will be collected as per the time points outlined in table 2 schedule of events

Study Visit If participant remains intubated	T0 +36h (±6h)	T0 +48h (±4h)	T0 +72h (±12h)	T0 +96h (±12h)	T0 +7d (±2d)	T0 +14d (±2d)	T0 +21d (±2d)	T0 +28d (±2d)	36w PMA (±1d)	40w PMA or Hospital Discharge (±1w)
Vital signs	*	*	*	*	*	*	*	*	*	*
Oxygen concentration	*		*	*	*	*	*	*	*	*
Ventilator modality	*	*	*	*	*	*	*	*	*	*
Ventilator settings	*	*	*	*	*	*	*	*	*	*
Haematology (SoC)		*	*	*	*					
Biochemistry (SoC)		*	*	*	*					
Plasma cytokine levels		*	*	*	*				*	
Plasma SP-D and rfhSP-D		*	*	*	*				*	
Blood gases		*	*	*	*				*	
ET cell counts	*	*	*	*	*	*	*	*		
ET rfhSP-D and SP-D levels		*	*	*	*				*	
Concomitant medication	*	*	*	*	*	*	*	*	*	*

Cranial ultrasound scan		*			*	*	*	*	*	*
Walsh Oxygen Test									*	
Review of AEs and SAEs	*	*	*	*	*	*	*	*	*	*
Review of DLEs	*	*	*	*						

Table 2. Subsequent time points in participant timeline following IMP administration. SoC: as per standard of care; ET: endotracheal aspirate (if remains intubated)

The RESPONSE study will collect clinical data and biological specimens (blood, tracheal and gastric aspirates) as per table 1 and 2. Parents/guardians/those legally responsible for the participant will have the option to give consent for any anonymised data and samples that are collected as part of RESPONSE to be used in other ancillary studies that have ethical approval. Gastric secretions will be taken from all infants at the time of admission after placement of oro/naso-gastric tube and will be discarded if consent is not obtained. Blood samples will be collected at birth, 12 hours, 24 hours, 48 hours, 72 hours, 96 hours, day 7 and at 36 weeks postmenstrual age.

Data collection and management

Participants once recruited to the RESPONSE study will be allocated a study number so that all data and samples that are taken are anonymized e.g RES_001. Participation in the clinical study will be recorded in the participant clinical records. Participants will be enrolled by the study team on the OpenClinica database. Participant clinical and laboratory data will be entered directly into the password protected study database. After completion of the trial the data will be exported and retained in restricted folders by the sponsor. All data will be held for 10 years following the completion of the trial.

Primary outcome data collection in this study (safety profile of rfhSP-D) will be done through grading and analysis of the incidence of DLEs. Potential causality of the DLE to the IMP will be assessed by an independent neonatologist. In addition to the dose-limiting events the following datasets will be collected from electronic patient records:

- At screening and on eligibility assessment: sex of participant, ethnicity, maternal medical history, antenatal steroid courses, date and time of rupture of membranes, concerns about maternal sepsis, ventilatory requirement on admission and administration of standard exogenous surfactant.

Secondary outcome data collection:

- Data will also be collected at the time points specified in the schedule of events (Tables 2 and 3) and this will include: concomitant medication, ventilatory support and parameters, known positive microbiology, use of postnatal steroids, presence and treatment of pulmonary hypertension, pneumothorax, patent ductus arteriosus, use of postnatal steroids.
- At 36 weeks PMA all participants will be assessed for severity of BPD and data will be collected about complications of prematurity such as episodes of necrotizing enterocolitis and retinopathy of prematurity. The severity of BPD in participants will be defined as per the 2019 NICHD criteria (36), an oxygen reduction test will be done if the participant is eligible (requiring less than Fi02 < 0.3 or 1.1L/min and not on positive pressure support) and remains an inpatient at the recruiting site.

All data will be handled in accordance with the Data Protection Act 2018 and GDPR and all study members will have current GCP training and certification.

Analysis of biological samples

Biological samples will be collected as per the schedule of events. Samples will be labelled with the participant's study number and transported to the Targeted Lung Immunotherapy Laboratory, UCL. Surfactant components, inflammatory markers and level of SP-D will be analysed using the ELISA technique (ELLA, BioTechne) with single marker studies and multiplex assays. Cytokines to be analysed include IL-1β, IL-6, IL-8, IL-11, IL-10, IL-13, matrix metalloproteinase-9 and tumour-necrosis factor-α. Cell counts (lymphocytes, neutrophils and macrophages) in gastric and tracheal aspirates will be assessed using flow cytometry. Samples will be retained if consent has been given by the parent/legal guardian for 5 years for use in any other ethics-approved studies. If consent is not given for use in further studies or at the end of 5 years, the biological samples will be destroyed as per the laboratory standard operating procedure.

Sample size and Statistical analysis

As this is a safety study, no formal sample size calculation has been performed. A sample size of 24 infants is planned to meet practical recruitment and time targets and to collect sufficient data to quantify the estimated risk of DLE at each dose level. Participants with unclear safety outcomes or who have not started study treatment will be replaced to meet our planned effective sample size of 24 participants.

The primary outcome of interest is the occurrence of DLEs at the dose levels under investigation and the identification of the dose(s) that, for infants of particular risk profiles defined by gestational age, have an estimated risk of DLE closest to the target side effect level of no greater than 20%. The use of Bayesian methodology to estimate risks will allow information to be borrowed across dose levels, making the dose-escalation and RP2D identification procedure more efficient than a standard rule-based approach.

The operating characteristics of the design, for three specific scenarios, are shown in Table 3. The first scenario is one where the initial a-priori DLE probabilities calculated by calibration (halfwidth of the indifference interval set at 0.05) correspond to the true underlying probabilities of DLE. The second scenario is such that the true DLE rate of the second dose level corresponds to the target DLE rate. The third scenario is one where the true probabilities are much lower than the a-priori probabilities.

Starting dose level 1, dose-skipping not allowed, 3 dose levels, maximum 24 participants.
Skeleton (a-priori probabilities of DLE) = 0.05, 0.11, 0.20
Target DLE rate = no greater than 20%

	Dose level		
	1	**2**	**3**
Scenario 1; recommendation [%]	2.5	24.5	73.0
Scenario 2; recommendation [%]	22.5	46.5	31.0
Scenario 3; recommendation [%]	0.0	3.3	96.7

Scenario 1: true probabilities = a-priori probabilities
Scenario 2: true probabilities = 0.10, 0.20, 0.30
Scenario 3: true probabilities = 0.02, 0.05, 0.10

Table 3. Operating characteristics of the Continuous Reassessment Model

Type and grade of DLEs, SAEs and AEs will be tabulated per dose level, and further summarised by risk group defined by gestational age. Mean estimated risk of DLE per dose level and 95% credibility intervals will be calculated using the study model. Secondary objectives will be described per dose level and risk category. Categorical variables will be summarised by frequencies and percentages, and continuous variables by means/medians and standard deviation/interquartile ranges per dose level.

Interim analyses of dose-limiting events

Interim analysis will done to assess if DLEs have occurred after cohorts of 3 participants at each dose level and data will be reviewed by the DSMB. CCTU will verify the participant data for the sentinel baby in this study 72 hours after the third dose of IMP is administered. If the first baby recruited to the study does not

receive all three doses, they will continue to have data collected for safety analysis but will not qualify as the sentinel baby for this study. For the remainder of the study interim analysis will be done after cohorts of three participants are recruited at each dose level to assess the occurrence of DLE and review all clinical data. The trial statistician will calculate and provide updated summaries of the estimate risk of dose-limiting toxicity at each dose level. The DSMB will then advise if dose escalation can occur. There will be no interim analysis for the secondary outcomes.

The study will be terminated if any of the following stopping rules are satisfied:

- There is at least 90% chance that the risk of DLE at dose level 1 is greater than the target of 20%. If the trial is terminated under this rule, no drug dose will be recommended due to safety concerns.
- The number of participants that have been treated without side effects is deemed sufficient.
- There is evidence of increased mortality or morbidity in the participants treated with the IMP.

Study Oversight and Monitoring

The sponsor will provide trial oversight and verify the trial processes and prompting corrective action to the clinical study team as required. An independent TSC will provide oversight of the trial to safeguard the interests of the trial participants. The TSC will also provide advice to the chief investigator (CI), CCTU and the funder on all aspects of the trial through its independent chair. An independent DSMB is assigned with an allocated chair. The DSMB will be responsible for monitoring and accumulating the safety data and making recommendations to the TSC on whether the trial should continue as planned. The DSMB will consider data as per the statistical analysis and make recommendations to the TSC chair for consideration by the TSC.

Patient and Public Involvement:

The TSC has a patient representative and the patient-facing documents have been reviewed and commented on by the patient representatives.

Adverse event reporting

All adverse events grade 3 or above on the NAESS/ SAE will be reported to CCTU within 24 hours until the participant reaches Day 7 following the last administration of the IMP (pre-clinical studies have demonstrated

that the IMP is not detectable in plasma sample taken 24 hours after final administration). All related events that are graded 1 or 2 according to the NAESS will be reported within 7 days. After Day 7 any events that are considered related to the IMP will be reported within 24 hours of knowledge to the sponsor. Assessments for, and reporting of all adverse events related/unrelated will continue until 40 weeks PMA or hospital discharge. All aggregated adverse events data will be considered by the DSMB at each meeting to confirm that there are no trends, safety signals or safety concerns. Examples of adverse events that are exempt from reporting are those that are graded 1 or graded 2 according to the NAESS criteria if considered not related to the IMP. These are common observations in pre-term infants and do not require a change in clinical management unless sustained, i.e. grade 3 and above on the NAESS. There is no formal frequency of audit for this study and but will be overseen by the sponsor and if required by MHRA.

Significance of Study

Despite the medical advances in neonatal medicine, the incidence of BPD has not changed over the years, and one may argue that it has increased because we see an increasing number of extremely preterm infants survive to discharge. The life-long morbidity associated with BPD has significant implications for healthcare systems around the world.

Infants at highest risk of BPD are born at a gestational age when the majority of the alveolar and vascular development in the lungs occurs. Immaturity of the lungs means they have a developmental deficiency of surfactant leading to RDS. Ongoing lung injury secondary to postnatal insults such as infection and mechanical ventilation leads to ongoing interruption to lung development. Efforts have been made over the year to reduce these insults by changes in ventilation strategies, early nutrition, and proactive management of infection in the hope that the lungs will repair and remodelling will lead to recovery of the lung parenchyma. However, a significant number of preterm infants will have multiple insults and despite best efforts will have abnormal repair with little lung recovery leading to BPD. Inflammation remains at the centre of the pathophysiology of BPD and the most promising target for therapies. Given this, there is a need for novel anti-inflammatory therapies to be explored such as SP-D.

The role of SP-D in lung immune homeostasis is well established but due to its propensity to oligomerise does not lend itself well to a stable drug form. The proposed recombinant fragment of SP-D has been developed into a stable drug form for endotracheal administration and animal studies in a well-established translational model have demonstrated its potential anti-inflammatory effects. This phase I safety study using dose

escalation of 1-4mg/kg will aim to identify a recommended Phase 2 dose for a subsequent randomised Phase 2 study in preterm infants born at less than 28 weeks gestation who are at highest risk of developing neonatal chronic lung disease.

Ethics and Dissemination

All results and analysis from the study will be published in peer-reviewed journals and presented at national and international conferences. All data generated in this study will be anonymised and the study will be conducted per Good Clinical practice. Access to the full study protocol will be given upon request and participant-level data will only be given if authorised by the sponsor for auditing purposes. Any substantial or non-substantial amendment to the study must be approved by the Health Research Authority (HRA) and will be communicated with the NHS trust research and development team to ensure site implementation. Any study material that is related to participant information or informed consent will be submitted to the principal research ethics committee for approval. This study has been approved by London-Brent NHS Research Health Authority ethics committee (REC reference 23/LO/0381) and has clinical trials approval (CTA 20363/0453/001-0001) in place.

Abbreviations

AE: Adverse Events

AR: Adverse Reaction

BAL: Bronchoalveolar Lavage

BPD: Bronchopulmonary dysplasia

CCTU: Comprehensive Clinical Trials Unit

CRM: Continual reassessment method

CTCAE: Common Terminology Criteria for Adverse Events

DLE: Dose Limiting Event

DSMB: Data and Safety Monitoring Board

GA: Gestational Age

HRA: Health Research Authority

IL: Interleukin

IMP: Investigational Medicinal Product

MHRA: Medicine and Healthcare products Regulatory Agency

MTD: Maximum Tolerated Dose

NAESS: Neonatal Adverse Event Severity Score

PMA: Post Menstrual Age

rfhSP-D: Recombinant Fragment of Human Surfactant Protein D

RP2D: Recommended phase 2 dose

SAE: Serious Adverse Event

SP: Surfactant Protein

TSC: Trial Steering Committee

UCL: University College London

Declarations of interest: None to declare by any of the authors.

Acknowledgements

We are grateful to our funders, the Medical Research Council for their ongoing support with this study. We would like to thank the UCL CCTU and the UCL/UCLH Joint Research Office team for their continued support and contribution in RESPONSE trial.

Authors' contributions

All authors contributed to the study design and ethics application. RB, HC, JM and NM wrote and edited the clinical trial protocol. All authors have reviewed and approved the manuscript.

Competing interests

The authors declare they have no competing interests.

References

1. Abman SH, Bancalari E, Jobe A. The Evolution of Bronchopulmonary Dysplasia after 50 Years. American Journal of Respiratory and Critical Care Medicine. 2017;195(4):421-4.
2. JOBE AH, BANCALARI E. Bronchopulmonary Dysplasia. American Journal of Respiratory and Critical Care Medicine. 2001;163(7):1723-9.
3. Siffel C, Kistler KD, Lewis JFM, Sarda SP. Global incidence of bronchopulmonary dysplasia among extremely preterm infants: a systematic literature review. The Journal of Maternal-Fetal & Neonatal Medicine. 2021;34(11):1721-31.

4. Thébaud B, Goss KN, Laughon M, Whitsett JA, Abman SH, Steinhorn RH, et al. Bronchopulmonary dysplasia. Nature Reviews Disease Primers. 2019;5(1):78.
5. Bancalari E, Claure N, Sosenko IRS. Bronchopulmonary dysplasia: changes in pathogenesis, epidemiology and definition. Seminars in Neonatology. 2003;8(1):63-71.
6. Ehrenkranz RA, Walsh MC, Vohr BR, Jobe AH, Wright LL, Fanaroff AA, et al. Validation of the National Institutes of Health consensus definition of bronchopulmonary dysplasia. Pediatrics. 2005;116(6):1353-60.
7. Davidson LM, Berkelhamer SK. Bronchopulmonary Dysplasia: Chronic Lung Disease of Infancy and Long-Term Pulmonary Outcomes. Journal of Clinical Medicine. 2017;6(1):4.
8. Zysman-Colman E, Tremblay G, Bandeali S, Landry S. Bronchopulmonary dysplasia - Trends over three decades. Paediatrics & child health. 2013;18:86-90.
9. Stoll BJ, Hansen NI, Bell EF, Walsh MC, Carlo WA, Shankaran S, et al. Trends in Care Practices, Morbidity, and Mortality of Extremely Preterm Neonates, 1993-2012. Jama. 2015;314(10):1039-51.
10. Costeloe KL, Hennessy EM, Haider S, Stacey F, Marlow N, Draper ES. Short term outcomes after extreme preterm birth in England: comparison of two birth cohorts in 1995 and 2006 (the EPICure studies). BMJ : British Medical Journal. 2012;345:e7976.
11. Hurst JR, Beckmann J, Ni Y, Bolton CE, McEniery CM, Cockcroft JR, et al. Respiratory and Cardiovascular Outcomes in Survivors of Extremely Preterm Birth at 19 Years. American Journal of Respiratory and Critical Care Medicine. 2020;202(3):422-32.
12. Um-Bergström P, Hallberg J, Pourbazargan M, Berggren-Broström E, Ferrara G, Eriksson MJ, et al. Pulmonary outcomes in adults with a history of Bronchopulmonary Dysplasia differ from patients with asthma. Respiratory Research. 2019;20(1):102.
13. Marlow N, Johnson S, Hurst JR. The extremely preterm young adult - State of the art. Semin Fetal Neonatal Med. 2022;27(3):101365.
14. Taghizadeh A, Reynolds E. Pathogenesis of bronchopulmonary dysplasia following hyaline membrane disease. The American journal of pathology. 1976;82(2):241.
15. Jobe AJ. The New BPD: An Arrest of Lung Development. Pediatric Research. 1999;46(6):641-.
16. Schultz C, Temming P, Bucsky P, Göpel W, Strunk T, Härtel C. Immature anti-inflammatory response in neonates. Clinical and experimental immunology. 2004;135(1):130-6.
17. Sullivan G, Galdi P, Borbye-Lorenzen N, Stoye DQ, Lamb GJ, Evans MJ, et al. Preterm Birth Is Associated With Immune Dysregulation Which Persists in Infants Exposed to Histologic Chorioamnionitis. Frontiers in Immunology. 2021;12.
18. Speer CP. Inflammation and bronchopulmonary dysplasia: A continuing story. Seminars in Fetal and Neonatal Medicine. 2006;11(5):354-62.
19. Possmayer F, Yu SH, Weber JM, Harding PG. Pulmonary surfactant. Can J Biochem Cell Biol. 1984;62(11):1121-33.
20. Watson A, Madsen J, Clark HW. SP-A and SP-D: Dual Functioning Immune Molecules With Antiviral and Immunomodulatory Properties. Frontiers in Immunology. 2021;11.
21. Wright JR. Immunoregulatory functions of surfactant proteins. Nature Reviews Immunology. 2005;5(1):58-68.
22. Beresford MW, Shaw NJ. Bronchoalveolar lavage surfactant protein a, B, and d concentrations in preterm infants ventilated for respiratory distress syndrome receiving natural and synthetic surfactants. Pediatr Res. 2003;53(4):663-70.
23. Kotecha S, Davies PL, Clark HW, McGreal EP. Increased prevalence of low oligomeric state surfactant protein D with restricted lectin activity in bronchoalveolar lavage fluid from preterm infants. Thorax. 2013;68(5):460-7.
24. Mackay R-MA, Townsend JP, Calvert J, Anthony M, Wilkinson AR, Postle AD, et al. Increased surfactant protein-D levels in the airways of preterm neonates with sepsis indicated responses to infectious challenges. Acta Paediatrica. 2019;108(5):870-6.
25. Clark H, Reid K. The potential of recombinant surfactant protein D therapy to reduce inflammation in neonatal chronic lung disease, cystic fibrosis, and emphysema. Archives of Disease in Childhood. 2003;88(11):981-4.

26. Clark H, Palaniyar N, Strong P, Edmondson J, Hawgood S, Reid KB. Surfactant protein D reduces alveolar macrophage apoptosis in vivo. J Immunol. 2002;169(6):2892-9.
27. Sato A, Whitsett JA, Scheule RK, Ikegami M. Surfactant Protein-D Inhibits Lung Inflammation Caused by Ventilation in Premature Newborn Lambs. American Journal of Respiratory and Critical Care Medicine. 2010;181(10):1098-105.
28. Knudsen L, Ochs M, Mackay R, Townsend P, Deb R, Mühlfeld C, et al. Truncated recombinant human SP-D attenuates emphysema and type II cell changes in SP-D deficient mice. Respir Res. 2007;8(1):70.
29. Sorensen GL, Hoegh SV, Leth-Larsen R, Thomsen TH, Floridon C, Smith K, et al. Multimeric and trimeric subunit SP-D are interconvertible structures with distinct ligand interaction. Mol Immunol. 2009;46(15):3060-9.
30. Strong P, Reid KBM, Clark H. Intranasal delivery of a truncated recombinant human SP-D is effective at down-regulating allergic hypersensitivity in mice sensitized to allergens of Aspergillus fumigatus. Clinical and experimental immunology. 2002;130(1):19-24.
31. Various A. Selected Abstracts of the 12th International Congress of UENPS; Krakow (Poland); September 2nd-4th, 2022. Journal of Pediatric and Neonatal Individualized Medicine (JPNIM). 2022;12(1):e120104.
32. O'Quigley J, Pepe M, Fisher L. Continual reassessment method: a practical design for phase 1 clinical trials in cancer. Biometrics. 1990;46(1):33-48.
33. Faries D. Practical modifications of the continual reassessment method for phase i cancer clinical trials. Journal of Biopharmaceutical Statistics. 1994;4(2):147-64.
34. Goodman SN, Zahurak ML, Piantadosi S. Some practical improvements in the continual reassessment method for phase I studies. Stat Med. 1995;14(11):1149-61.
35. Salaets T, Turner MA, Short M, Ward RM, Hokuto I, Ariagno RL, et al. Development of a neonatal adverse event severity scale through a Delphi consensus approach. Arch Dis Child. 2019;104(12):1167-73.
36. Jensen EA, Dysart K, Gantz MG, McDonald S, Bamat NA, Keszler M, et al. The Diagnosis of Bronchopulmonary Dysplasia in Very Preterm Infants. An Evidence-based Approach. American Journal of Respiratory and Critical Care Medicine. 2019;200(6):751-9.

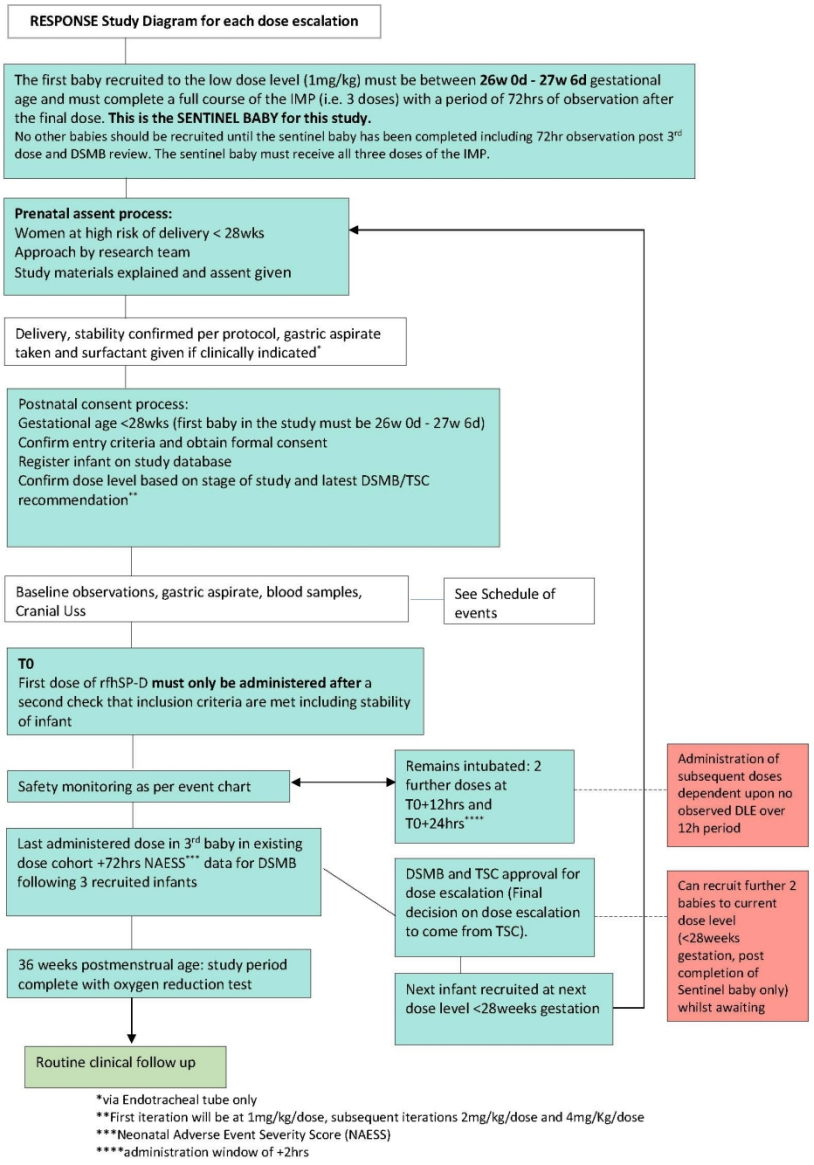
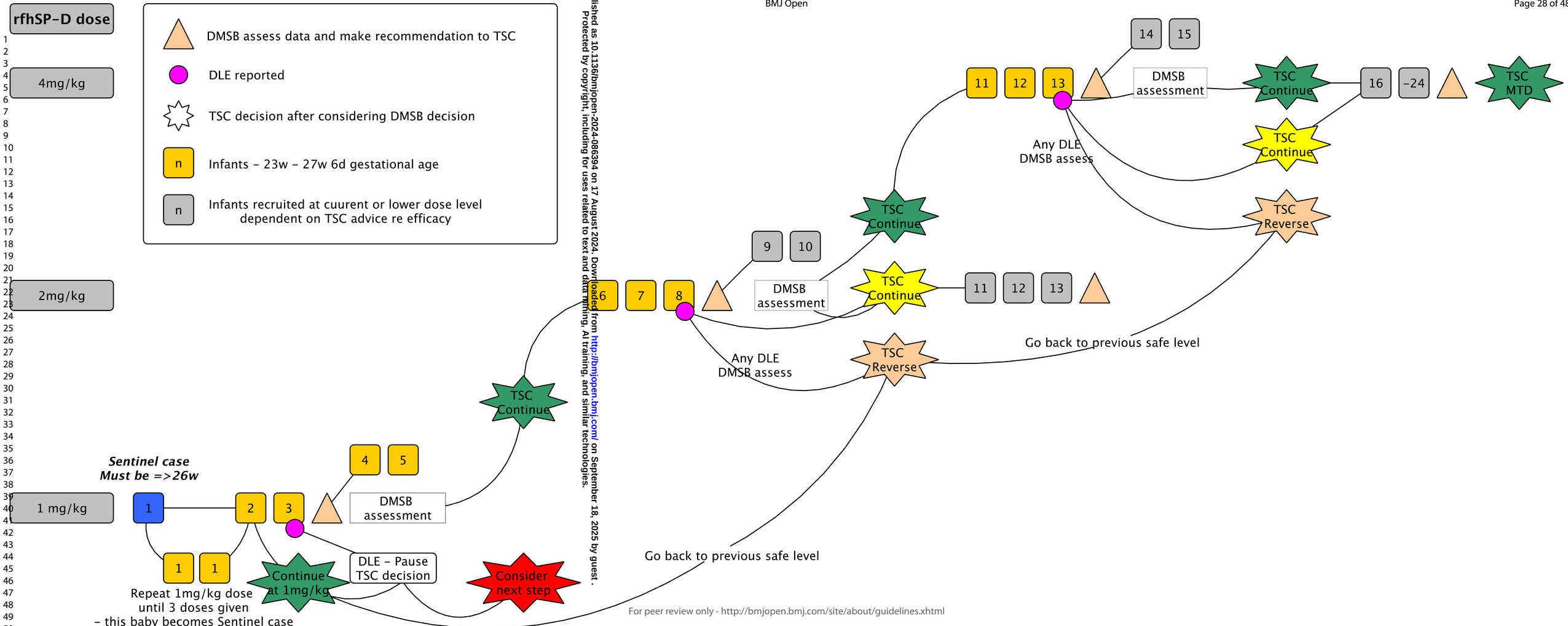


Figure 1. Dose escalation process in RESPONSE using rfhSP-D in preterm infants at risk of BPD.

209x297mm (200 x 200 DPI)



University College London Hospitals NHS
NHS Foundation Trust

UCL
COMPREHENSIVE CLINICAL TRIALS UNIT

RESPONSE Clinical Study Patient Information Sheet

We are inviting your baby to take part in a first in human clinical study titled – RESPONSE: Recombinant **s**urfactant **pro**tein D (rfhSP-D) to prevent **n**eonatal chronic lung disea**se**

- Please take time to read the following information carefully. Before you decide we would like you to understand why the research is being done and what this involves for you and your baby.
- Discuss it with friends and relatives if you wish.
- If you choose not to take part, this will not affect the care your baby receives in any way.
- Your baby can stop taking part in the study at any time, without you needing to give a reason.
- Ask us if there is anything that is not clear, or if you would like more information.

Important things that you need to know

- We are inviting parents of all babies born prematurely before 28 weeks gestation to help with this study.
- This is the first time this medication has been used in humans.
- This medication will be given as extra and not instead of usual surfactant therapy.
- We want to find out whether giving an additional surfactant protein D (a protein our body produces naturally) to premature babies will prevent them from developing chronic lung disease, also known as Bronchopulmonary dysplasia [BPD].
- The goal of the study is to establish the best dose of an artificial surfactant protein D (rfhSP-D) for preterm babies.
- This is the first study to use a man-made surfactant protein D, which is a protein that is naturally made by our bodies.

If your baby is enrolled into the study, they will continue to receive the standard neonatal care that we provide for all premature babies and this includes standard surfactant therapy that helps your baby's lungs when they are first born.

Contents:

How to contact us

If you have any questions about this trial, please talk to your study doctor or nurse:

Dr Reena Bhatt
Neonatal Unit
Elizabeth Garret Anderson Building, Level 2
Grafton Way
Mob: 07930288004

1 Why are we doing this study?

Bronchopulmonary Dysplasia (BPD), also known as Chronic Lung Disease (CLD) can be a problem for lots of babies born before 28 weeks of pregnancy. This study aims to use a naturally occurring protein to help prevent premature babies from developing CLD.

What is Neonatal Chronic Lung Disease?

CLD is a serious, long-term lung condition that can affect up to 70% of babies born prematurely before 28 weeks of pregnancy. This is because their lungs have not fully developed, and they do not produce an important substance called Surfactant for several days after birth. CLD is a condition where babies need extra oxygen for a long time after they are born. The reason why they develop CLD is not clear, but we do know that inflammation and infection have a role. Most babies with CLD get better, but some are particularly vulnerable to other problems, and it may affect their long-term development and lung health.

What is surfactant?

Surfactant is a naturally occurring soapy substance, produced in the lungs, which makes it easier to breathe and prevents the lungs from collapsing. It also has a role in protecting the lungs from infection and inflammation. Very premature babies have lungs that do not produce surfactant or only have small amounts and we routinely give such babies replacement therapy. Premature babies are given extra surfactant directly into their lungs soon after birth and this has greatly improved their survival. Naturally occurring surfactant is a complex substance containing a soap like substance and 4 proteins that are called surfactant A, B, C and D. The surfactant replacement treatment we use only contains surfactant proteins B and C.

What is surfactant protein D?

Laboratory studies have shown that surfactant protein D is important in preventing inflammation and infection in the lungs, which cause lung tissue to become swollen and makes providing care more difficult, prolonging the baby's illness. We have been able to make in the laboratory a version of surfactant protein D which is the important active part of the whole protein. In the laboratory this reduces signs of inflammation and prevents infection, and it appears safe and effective.

What are we trying to find out?

We now wish to use this new protein to help babies who need to receive surfactant replacement treatment in the neonatal unit. The purpose of this study is to look at what the safest dose of surfactant protein D is and how it helps to prevent CLD in very premature babies who are given surfactant replacement therapy. The goal of the study is to find the recommended dose of surfactant protein D for premature babies while keeping them safe.

2 Why is my baby being asked to take part?

Your baby is invited to take part in the RESPONSE study for these reasons:

- Your baby was or is likely to be born at less than 28 weeks of gestation (number of weeks of pregnancy).
- Your baby will be admitted to the neonatal unit and will be receiving surfactant therapy and is likely to need breathing support.
- Your baby is at risk of developing CLD.

3 What do I need to know about the treatments used in this study?

Surfactants are naturally occurring proteins made in the lungs that help make sure the lungs don't collapse. Premature babies do not produce surfactant or may not have enough, as described above.

In this study we will be using a small fragment of this naturally occurring protein and giving it straight into babies' lungs in exactly the same way as we do with standard surfactant replacement treatment.

4 What will I need to do if I want my baby to take part?

Can my baby definitely take part?

Not all babies that are born before 28 weeks will be able to take part in the study. The study has participation criteria to ensure that we do the safest thing for your baby.

If your baby meets the study requirements, you will be approached by the study team and be given information on the study. You will have the opportunity to ask any questions you may have regarding the study.

What if my baby meets the study entry requirements?

If your baby meets all the entry requirements for the RESPONSE study, the research nurse/doctor who is part of the neonatal unit team will go through the next key steps with you.

We will ask you to give us your consent by signing a form that a member of the team will go through.

What will happen to my baby during the study?

If you agree to take part in the study, you will be asked to sign a consent form. A member of the study team will then go through the initial assessments required for the study including collecting details of your medical history, pregnancy history and any medications that you are taking or may be given in pregnancy and labour.

We have included a table at the end of this information pack to outline the steps that will be taken in this study. Please be assured that if your baby is or is not participating in this study, they will still receive the high level of neonatal care that is standard at UCLH.

Stomach Secretions:

All premature babies admitted to the unit have a plastic tube that is passed into their stomach from their nose called a nasogastric [NG] tube. The purpose of this tube is to remove air and secretions from the stomach and to give milk feeds. When the NG tube is first passed the secretions usually consist of the fluid from the womb and are usually discarded once they have been tested to see if the NG tube is placed correctly in the stomach. We intend to use some of these secretions for our research to check the levels of cells that are part of the immune system and the levels of surfactant protein D.

Lung Secretions:

A baby that needs help with their breathing from a ventilator will have a tube that sits in the large airways [windpipe/trachea], called an endotracheal tube [ET tube]. This connects the ventilator to the baby's lungs. To allow the ventilator to work effectively, the tube and the baby's large airways are cleared regularly of secretions by gentle suctioning by the neonatal team. Normally these secretions are thrown away. We intend to use them for our investigations to see the effect of the surfactant protein D that your baby will receive.

The lung secretions will be collected as outlined in the sample table at the end of this document only if your baby is on a ventilator and is intubated.

Blood Samples:

All premature babies have regular blood tests, especially in the first week after birth. To allow blood pressure monitoring and blood tests and to give nutrition, most premature

babies will have small catheters inserted into the large blood vessels present in their umbilical cords, these are called umbilical venous and arterial catheters [UVCs and UACs].

Once you have given consent for your baby to take part in the study, an extra blood sample along with the regular standard-of-care blood samples will be taken. The extra blood sample is to look at the levels of the drug and other proteins in your baby's blood.

We expect your baby to have a maximum of 7 samples as part of this study. The blood samples are very small, it would take 10 of these samples to fill a teaspoon and is unlikely to lead to a need for blood transfusion.

All blood samples will be, when possible, taken at the same time as routine blood tests. The blood samples will be temporarily stored in a fridge in the hospital neonatal unit, before being transferred to our central laboratory in the UCL EGA Institute for Women's Health, where it processed, stored, and analysed.

With your consent, the left over samples will be kept for up to 5 years and may be used in other research that has been approved by the Research Ethics Committee. However, if you do not consent for the samples to be stored and analysed, please let the clinical team know so that the samples can be destroyed.

Recombinant Surfactant Protein D [rfhSP-D]:

The surfactant protein D is made into a liquid that is similar to that of the standard surfactant therapy that is given – this is called rfhSP-D. Your baby, depending on which stage of the study they are enrolled, will receive up to three doses of either 1mg/kg, 2mg/kg or 4mg/kg of rfhSP-D.

Babies will be enrolled in groups, with dose increase in each group only after it has been deemed safe to increase the dose by the clinical investigator, the doctor responsible for the baby's care and an independent group of professionals who form our Drug Safety Monitoring Board, who have nothing to do with the clinical care your baby receives.

Recombinant Surfactant Protein D [rfhSP-D] will be given to your baby three times. Firstly, 2 hours after birth, then again after 12 hours, and finally after 24 hours. The drug will be given by the same method that we use to give standard surfactant therapy, which is by using a fine tube passed into the baby's breathing tube, which allows the drug to be given straight into the lungs. Your baby will then continue to receive our standard care in the Neonatal Unit until discharge. If your baby does not have a breathing tube before all the doses are given then they will not be given any further doses and will only be monitored to see how they are progressing.

Please refer to the summary of procedures at the end of this document for further information.

5 What are the possible benefits of taking part?

We hope that your baby will be helped by being treated in this study, but this cannot be guaranteed.

The information we get from this study will help us to improve treatment for future babies with BPD.

6 What are the possible side effects?

What are the most common side effects?

This is a safety study using rfhSP-D to assess possible side-effects. Most babies that are born prematurely, especially before 28 weeks, will need surfactant replacement treatment This is usually given through a thin tube that is passed into the breathing tube, and then taken out. Giving the surfactant including the drug in this study this way is most effective as we know that it goes straight to the lungs where it needs to work.

The drug that is part of the study will be given in the same way that we give the standard surfactant. We know that the protein in the study drug is a naturally occurring protein, we are not expecting any significant side-effects. There may be some side-effects from giving extra liquid into the lungs and through the breathing tube. These may include:

- Blocking the breathing tube
- Causing the heart rate to go down briefly
- Causing the oxygen level to go down briefly

We sometimes see these effects when we give surfactant as part of our care, and they usually recover quickly as the liquid is absorbed quickly in the lungs. If we are concerned at all we would stop giving the medication.

We are not aware of any other side-effects of the study drug. The study drug has been carefully studied in laboratory models that mimic the condition in humans. We know from these studies that when we have given the medication through a breathing tube, no side effects have been seen straight after or up to 2 weeks after the medication is given. In the laboratory we collected measurements of heart rate and breathing support, we looked at their tissues and the levels of the drug and other proteins that cause inflammation of the lungs. We found no effect on any of the measurements we took. We saw that the drug is cleared from the lungs quickly and inflammation is less than expected.

If you would like to know more about the results of the laboratory studies please ask one of the study team who can explain them further for you.

7 What are the possible disadvantages and risks of taking part?

RESPONSE is a dose safety study. The medicine is unlicensed and has not yet been administered to humans but has been extensively studied in animal models. The Medicines Health Regulatory Body [MHRA] have reviewed this medication and, as it is a naturally occurring protein, do not feel it needs studies in adult patients and have authorised for its first use in premature infants.

All medical procedures involve the risk of harm, but this is usually a low risk. In addition, there might be risks associated with this study that we do not yet know about as this is a safety study. Animal toxicology studies were done using this drug and no toxic effects were found at the doses that we are using or at higher doses. If you have questions about side-effects, please ask your study doctor.

New information about the treatment being studied may become available while the study is running. We will tell you about any new findings that might affect your decision about your baby's continuation in the study.

8 More information about taking part

Does my baby have to take part in the RESPONSE study?

No, it is up to you to decide whether or not to take part. If you decide to take part, you will be given this information sheet to keep and you will be asked to sign a consent form.

A decision not to take part will not affect the standard of care you receive.

The Patient Advice and Liaison Service (PALS) offers confidential advice, support, and information on health-related matters. You have the opportunity to discuss any concerns about the study or the care of your baby with a PALS officer in your hospital.

Expenses and Payments

These are not met by the study as we would not require either you or your baby to visit us beyond the time that your baby needs to recover after birth until discharge.

Please note that all discoveries (intellectual property) are a gift to UCL and that you will not benefit financially if the research leads to a new treatment.

Can I change my mind after my baby has joined the study?

You can change your mind about your baby's participation in the study at any time and without giving a reason, but you must talk to your study doctor first. They can advise you about any concerns you may have.

A decision to stop taking part at any time will not affect the standard of care your baby receives.

What will happen to the information collected about me and my baby during the study?

How will we use information about you?

We will need to use information from your infant from their medical records for this research project.

This information will include your infant's date and time of birth and your name and contact details. People will use this information to do the research or to check your records to make sure that the research is being done properly.

People who do not need to know who you are will not be able to see your name or contact details. Your data will have a code number instead.

We will keep all information about you safe and secure.

Once we have finished the study, we will keep some of the data so we can check the results. We will write our reports in a way that no-one can work out that you took part in the study.

UCLH (the hospital) will keep identifiable information about you for a minimum of 10 years after the study has finished.

The type of information collected about you and your baby are demographics (such as date/time of birth, birth weight etc.), pregnancy and delivery history, and any clinical assessments that are performed during the trial. If your baby's care is transferred to another hospital, we may need to contact this hospital so that we can continue to collect data and monitor your baby.

UCLH will keep your name and contact details confidential and will not pass this information to University College London. We will use this information as needed, to contact you about the research study, and make sure that relevant information about the study are recorded for your care, and to oversee the quality of the study. Certain individuals from University College London and regulatory organisations may look at your medical and research records to check the accuracy of the research study. The people who analyse the information will not be able to identify you and will not be able to find out your name or contact details.

What are your choices about how your information is used?

You can stop your infant being part of the study at any time, without giving a reason, but we will keep information about your infant that we already have.

If you choose for your infant to stop taking part in the study, we would like to continue collecting information about your infant's health from your hospital medical records. If you do not want this to happen, tell us and we will stop.

We need to manage your records in specific ways for the research to be reliable. This means that we won't be able to let you see or change the data we hold about your infant.

Your anonymised information could be used for future research in any aspect of health or care and could be combined with information about you from other sources held by researchers. However, ethics approval will be sought prior to use of this information, and no one is able to identify you or your baby from the data we use.

The RESPONSE trial may use the collected data for marketing authorisation to develop rfhSP-D as a licensed and commercial product for the future of other infants; however only anonymised data will be provided to the authorities.

UCL CCTU is registered under the provisions of the 2018 Data Protection Act (DPA) to store this information. There is a question about this on the consent form that we will ask you to sign before you begin the study.

Where can you find out more about how your information is used?

You can find out more about how we use your information:

- at www.hra.nhs.uk/information-about-patients/
- our leaflet 'How health researchers use information from participants in clinical trials' available from https://www.ucl.ac.uk/comprehensive-clinical-trials-unit/use-data
- https://www.ucl.ac.uk/legal-services/privacy/ucl-general-privacy-notice-participants-and-researchers-health-and-care-research-studies
- by asking one of the research team
- by sending an email to the UCL Data Protection Officer on data-protection@ucl.ac.uk
- by emailing us on cctu-enquiries@ucl.ac.uk

What will happen to the results of the RESPONSE study?

We will publish the results in a medical journal, so that other doctors can see them. We also work closely with patient groups to advertise the results of the study in an easy-to-read format for patients. You can ask your study team or usual doctor for a copy of any publication or link to patient websites when the study is published. Your identity and any

personal details will be kept confidential. No named information about you will be published in any report relating to this trial.

Who is organising and funding the trial?

This study is organised by UCL CCTU, which has run trials for many years. The study coordination, data collection and analysis and administration will be provided by UCL CCTU. You can find out more about us at https://www.ucl.ac.uk/cctu.

UCL is the sponsor for this study and has overall responsibility for the conduct of the study. They are responsible for ensuring the study is carried out ethically and in the best interests of the study participants.

This study is funded by the Medical Research Council Developmental Pathway Funding scheme.

Who has reviewed the RESPONSE study?

The study has been reviewed and authorised by the Medicines and Healthcare products Regulatory Agency (MHRA), as well as a Research Ethics Committee, the Health Research Authority (HRA), and the Research and Development Office at all participating hospitals.

What if new information becomes available during the course of the trial?

Sometimes during a study, new information becomes available about the treatment options being studied. If this happens, the study doctor will tell you about it and discuss with you whether you wish for your baby to continue the study. If you decide that your baby should stop taking part in the study, your doctor will arrange for your baby's care to continue outside of the study. However, if you decide that your baby should continue, you might be asked to sign an updated consent form.

Your doctor might also suggest that it is in your baby's best interest to stop taking part in the study. Your doctor will explain the reasons and arrange for your baby's care to continue outside the study. Your baby will continue to receive standard medical care.

What happens if the RESPONSE study stops early?

Very occasionally a study is stopped early. If this happens, the reasons will be explained to you. Your study doctor will arrange for your baby's care to continue outside of the study. Your baby will resume standard medical care.

What if something goes wrong?

Every care will be taken in the course of this clinical study.

Complaints

In the event that something does go wrong, and your infant is harmed during the research, and this is due to someone's negligence, then you may have grounds for a legal action for compensation against UCL/UCLH but you may have to pay your legal costs. The normal National Health Service complaints mechanisms will still be available to you.

Alternatively, you can contact the Patient and Advice Liaison Service at the hospital:

UCLH Patient Advice & Liaison Service (PALS)

Address: PALS

Ground Floor Atrium

University College Hospital

235 Euston Road

London NW1 2BU

Telephone (main hospital): 02034473042

Telephone (NHNN): 02034483237

You can also contact the UCLH Patient Advice and Liaison Service on 02034483237/02034473042 (as appropriate see above) or email Uclh.pals@nhs.net

9 Contacts for further information

If you want further information about the RESPONSE study, please contact the study doctor using the details provided on the front page of this Participant Information Sheet.

10 Glossary

Bronchopulmonary Dysplasia and Chronic Lung Disease: The need for oxygen or breathing support at 36 weeks corrected gestational age (this means when you would have been 36 weeks pregnant)

Surfactant: a soapy substance that is made in enough amounts by the lungs from 34 weeks gestation

Gestation: number of weeks of pregnancy

Endotracheal (ET) tube: this is a tube that goes in from the mouth into the windpipe (trachea).

Intubated: having a tube in the wind-pipe (trachea) and being connected to a breathing machine

Extubated: after the breathing tube (ET tube) is taken out

Nasogastric tube: a small thin tube that goes in from the nose into the stomach that helps your baby get medicines and milk

THANK YOU FOR TAKING THE TIME TO CONSIDER TAKING PART IN THE RESPONSE TRIAL.

Table of samples that your baby will have done as part of the study. Not all these will be done if your baby is not ventilated.

Time point after consent	T0 Hrs (the time of drug given)	T0+12 Hrs	T0+24 Hrs	T0+36 Hrs	T0+48 Hrs	T0+72 Hrs	T0+96 Hrs	T0+ Day 7	36 Wks post menstrual age
Stomach secretions	✓								
Lung secretions [only if intubated]		✓	✓	✓	✓	✓	✓	✓	✓
Blood sample	✓		✓		✓	✓	✓	✓	✓ *

**Sample will be taken if participant is still in hospital*

Reporting checklist for protocol of a clinical trial.

Based on the SPIRIT guidelines.

Instructions to authors

Complete this checklist by entering the page numbers from your manuscript where readers will find each of the items listed below.

Your article may not currently address all the items on the checklist. Please modify your text to include the missing information. If you are certain that an item does not apply, please write "n/a" and provide a short explanation.

Upload your completed checklist as an extra file when you submit to a journal.

In your methods section, say that you used the SPIRITreporting guidelines, and cite them as:

Chan A-W, Tetzlaff JM, Gøtzsche PC, Altman DG, Mann H, Berlin J, Dickersin K, Hróbjartsson A, Schulz KF, Parulekar WR, Krleža-Jerić K, Laupacis A, Moher D. SPIRIT 2013 Explanation and Elaboration: Guidance for protocols of clinical trials. BMJ. 2013;346:e7586

		Reporting Item	Page Number
Administrative information			
Title	#1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	#2a	Trial identifier and registry name. If not yet registered, name of intended registry	3
Trial registration: data set	#2b	All items from the World Health Organization Trial Registration Data Set	3
Protocol version	#3	Date and version identifier	3
Funding	#4	Sources and types of financial, material, and other support	3
Roles and responsibilities: contributorship	#5a	Names, affiliations, and roles of protocol contributors	1 and 28

Roles and responsibilities: sponsor contact information	#5b	Name and contact information for the trial sponsor	3
Roles and responsibilities: sponsor and funder	#5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	3
Roles and responsibilities: committees	#5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	22
Introduction			
Background and rationale	#6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	4
Background and rationale: choice of comparators	#6b	Explanation for choice of comparators	N/A this is a phase I study and there is no control or comparator.
Objectives	#7	Specific objectives or hypotheses	8
Trial design	#8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, non-inferiority, exploratory)	8
Methods: Participants, interventions, and outcomes			

Study setting	#9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	8
Eligibility criteria	#10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	12
Interventions: description	#11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	13
Interventions: modifications	#11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving / worsening disease)	14
Interventions: adherance	#11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return; laboratory tests)	14
Interventions: concomitant care	#11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	13/14
Outcomes	#12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	14
Participant timeline	#13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	16
Sample size	#14	Estimated number of participants needed to achieve study objectives and how it was	20

		determined, including clinical and statistical assumptions supporting any sample size calculations	
Recruitment	#15	Strategies for achieving adequate participant enrolment to reach target sample size	13
Methods: Assignment of interventions (for controlled trials)			
Allocation: sequence generation	#16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	n/a - this is an open-labelled safety trial. All participants will be anonymised with a study number that will be sequential as they are recruited to the study.
Allocation concealment mechanism	#16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	n/a – this is not a blinded study but a safety phase I study.
Allocation: implementation	#16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	18
Blinding (masking)	#17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	n/a - this is a Phase I safety study with only one intervention group and there will be no blinding of the intervention.
Blinding (masking): emergency unblinding	#17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	n/a - this is a Phase I safety study.

Methods: Data collection, management, and analysis			
Data collection plan	#18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	18
Data collection plan: retention	#18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	20
Data management	#19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	18
Statistics: outcomes	#20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	20
Statistics: additional analyses	#20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	n/a - there is only one group in this protocol and therefore no subgroup analyses will be done.
Statistics: analysis population and missing data	#20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	20

Methods: Monitoring			
Data monitoring: formal committee	#21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	22
Data monitoring: interim analysis	#21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	21
Harms	#22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	22
Auditing	#23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	22
Ethics and dissemination			
Research ethics approval	#24	Plans for seeking research ethics committee / institutional review board (REC / IRB) approval	23
Protocol amendments	#25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC / IRBs, trial participants, trial registries, journals, regulators)	23
Consent or assent	#26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	13

Consent or assent: ancillary studies	#26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	16
Confidentiality	#27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	19
Declaration of interests	#28	Financial and other competing interests for principal investigators for the overall trial and each study site	25
Data access	#29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	19
Ancillary and post trial care	#30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	15
Dissemination policy: trial results	#31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	23
Dissemination policy: authorship	#31b	Authorship eligibility guidelines and any intended use of professional writers	23
Dissemination policy: reproducible research	#31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	23
Appendices			
Informed consent materials	#32	Model consent form and other related documentation given to participants and authorised surrogates	Appendix 1
Biological specimens	#33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or	20

molecular analysis in the current trial and for future use in ancillary studies, if applicable

BMJ Open

Recombinant Surfactant protein D to prevent neonatal chronic lung disease (RESPONSE): a protocol for a phase I safety trial in a tertiary neonatal unit.

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2024-086394.R1
Article Type:	Protocol
Date Submitted by the Author:	24-May-2024
Complete List of Authors:	Bhatt, Reena; University College London Hospital Neonatal Intensive Care Unit; University College London, Neonatology Madsen, Jens; University College London, Department of Neonatology Castillo-Hernandez, Tania; University College London, Department of Neonatology Chant, Kathy; University College London, Department of Neonatology Dehbi, Hakim-Moulay; University College London Institute of Clinical Trials and Methodology Marlow, Neil; UCL Clark, Howard; University College London Hospitals; University College London, Department of Neonatology
Primary Subject Heading:	Paediatrics
Secondary Subject Heading:	Respiratory medicine, Research methods
Keywords:	IMMUNOLOGY, Neonatal intensive & critical care < INTENSIVE & CRITICAL CARE, RESPIRATORY MEDICINE (see Thoracic Medicine)

Title

Recombinant Surfactant protein D to prevent neonatal chronic lung disease (RESPONSE): a protocol for a phase I safety trial in a tertiary neonatal unit.

Names protocol contributors

Reena Bhatt[1,2], Jens Madsen[2], Tania Castillo-Hernandez[2], Kathy Chant[2], Hakim-Moulay Dehbi[3], Neil Marlow[2], Howard Clark[1,2]

[1] Neonatal Intensive Care Unit, EGA Wing, University College London Hospitals, London, United Kingdom

[2] Department of Neonatology, EGA Institute for Women's Health, University College London, London, United Kingdom

[3] Comprehensive Clinical Trials Unit, University College London, London, United Kingdom

Corresponding author

Dr Reena Bhatt

EGA Institute for Women's Health

Rockefeller Building

University College London

21 University Street

London, WC1E 6DE

United Kingdom

Tel: 02076796031

Email: reena.bhatt@ucl.ac.uk

Abstract

Introduction: Chronic respiratory morbidity from bronchopulmonary dysplasia (BPD) remains the most common complication of preterm birth and has consequences for later respiratory, cardiovascular and neurodevelopmental outcomes. The early phases of respiratory illness are characterised by rapid consumption of endogenous surfactant and slow replenishment. Exogenous surfactant is routinely administered to infants born before 28 weeks of gestation as prophylaxis.

Endogenous surfactant includes four proteins, known as surfactant proteins (SP) A, B, C and D. Current bovine- and porcine-derived surfactant preparations only contain surfactant proteins B and C. SP-D has a key role in lung immune homeostasis as part of the innate immune system. Laboratory studies using recombinant SP-D have demonstrated reduced inflammation, which may be a pathway to reducing the associated morbidity from BPD. RESPONSE utilises a recombinant fragment of human surfactant protein D (rfhSP-D), in a phase I safety and dose-escalation trial as the first stage in determining its effect in humans.

Methods and Analysis: This is a single centre, dose-escalation, phase I safety study aiming to recruit 24 infants born before 28 weeks gestation with respiratory distress syndrome (RDS). In addition to routine surfactant replacement therapy, participants will receive three doses of rfhSP-D via endotracheal route at either 1mg/kg, 2mg/kg or 4mg/kg. The study utilises a Bayesian Continual Reassessment Method (CRM) to make dose escalation decisions. Dose-limiting events (DLE) in this trial will be graded according to the published neonatal adverse event severity score (NAESS). The primary outcome of this study is to evaluate the safety profile of rfhSP-D across each dose level based on the profile of DLE to establish the recommended phase 2 dose (RP2D) of rfhSP-D.

Ethics and Dissemination: The RESPONSE study has received ethical approval. Results from the study will be published in peer-reviewed journals and presented at national and international conferences.

Trial registration: Medical EudraCT: 2021-001824-16, ISRCTN: 17083028, Clinical trials.gov.uk: NCT05898633.

Protocol Version: RESPONSE Protocol v3.0 25/01/2024

Name and contact information for the trial sponsor: University College London (UCL) with sponsor responsibilities delegated to the Comprehensive Clinical Trails Unit (CCTU). Contact:

cctu.response@ucl.ac.uk

Role of sponsor: Specific functions have been delegated to the UCL CCTU by the sponsor. A clinical project manager at the UCL CCTU will oversee the clinical trial manager who will be responsible for the day-to-day management of the trial. The CCTU staff will be involved in site initiation, database construction, development of the protocol and trial-related documentation. The sponsor will be responsible for the audit of the trial.

Key Words: Neonates, bronchopulmonary dysplasia, surfactant protein D, prematurity, Bayesian method

Article Summary:

Strengths:

- This study uses the International Neonatal Consortium (INC) Neonatal Adverse Event Severity Score (NAESS) which is specific to this population and allows a better grading and understanding of the adverse events and progression of the trial. This scoring system unlike others e.g. Common Terminology Criteria for Adverse events (CTCAE) takes into account age-appropriate behaviour e.g. feeding and physiological parameters such as changes in oxygenation. Although the NAESS has not been rigorously validated, it is well-placed to improve the quality of drug evaluation in this highly vulnerable population.
- This is a safety study aiming to establish a recommended phase II dose of a novel therapy in a highly vulnerable population affected by Bronchopulmonary dysplasia which has a significant impact on long-term lung health.
- This study utilises Bayesian analysis which utilises prior cohort data to inform the ongoing dose escalation.

Limitations:

- This is a single-centre study which may affect recruitment and the population characteristics.

Introduction

Clinical Need for Study

The introduction of exogenous surfactant replacement therapy has significantly improved mortality in extremely preterm infants, those born before 28 weeks of gestation (1). Despite this, chronic respiratory morbidity from BPD remains the most common complication of very preterm birth. BPD may be formally defined by the persisting need for respiratory support past 36 weeks postmenstrual age (PMA) (2). It affects up to 75% of extremely preterm infants (3), with decreasing prevalence with increasing gestational age (4). The pathogenesis of BPD is complex and multifactorial, involving lung immaturity, infection, inflammation, oxygen toxicity and ventilator-induced injury.

Unlike when first described, BPD is now rarely seen in infants born at more than 1200 g or after 30 weeks of gestation (2, 5, 6) due to the introduction of antenatal steroid administration, surfactant replacement therapy, improved ventilation strategies and better nutrition (7, 8). The prevalence of BPD has not fallen as expected (8-10). It can be argued that with advances in neonatal care leading to increased survival of infants at greatest risk of BPD, the prevalence may increase in years to come presenting a challenge for healthcare systems worldwide. Furthermore, BPD has lifelong consequences, with respiratory impairment that has important implications for adult clinicians, tracking through to adult life (11, 12) and neonatal BPD is also a marker for adult cognitive, educational and behavioural impairment with implications for health, wealth and relationships for life (13).

As the mean gestational age of neonatal populations has fallen with increasing survival, the pathophysiology of chronic respiratory disease in very preterm populations has changed. Whereas the original descriptions of BPD related the occurrence and progression primarily to barotrauma from mechanical positive pressure ventilation (14), with increasing immaturity the profile of causation has changed, and this “new” BPD (15) is primarily found among extremely preterm infants. The primary driver in its development is lung inflammation, subject to the other risks referred to above. The disease is characterised by developmental

arrest of lung tissue and a loss of alveolar septation by impairing alveolar crest development. This interruption in normal lung development with superimposed inflammation, oxygen toxicity and pressure-induced changes (barotrauma, volutrauma, atelectotrauma) completes the clinical picture.

Postnatal pulmonary inflammation is due to an imbalance in humoral factors favouring a pro-inflammatory response (16, 17) and increased presence of inflammatory cells in the airway (18). Inflammation, secondary to positive pressure ventilation, oxygen therapy or infection, may have further impact on the cytokine profile and the interruption of lung development. The overwhelming evidence for inflammation as a causal mechanism in the development of BPD suggests that early anti-inflammatory therapies might reduce the frequency and severity of the condition. Identification of potential therapeutic targets remains a goal to reduce the frequency of BPD in high-risk infants. Naturally occurring SP-D has gained increasing interest as a potential immunotherapy to dampen the pro-inflammatory cascade and facilitate lung repair, thus reducing the frequency and severity of lung disease. In turn, this may have important long-term benefits for the child.

Surfactant Protein D

Mammalian surfactant comprises largely phospholipids (80%), neutral lipids (10%) and surfactant proteins (10%), dipalmitoylphosphatidylcholine (DPPC) being the primary surface-active component at the alveolar surface (19). Four SP are found in surfactant, SP-A, SP-B, SP-C and SP-D. SP-B and SP-C are hydrophobic and their role is largely to stabilise the lipid monolayer formed at the air-liquid interface by stimulating phospholipid adsorption and reducing surface tension. Due to their hydrophobic nature, these SP are easily extracted from bovine or porcine sources and present in widely used commercial surfactants. In contrast, SP-A and SP-D are hydrophilic and are not present in the surfactant preparations currently used in clinical practice.

SP-D is an essential lung component and functions to keep the lungs in a hypo-responsive state at rest, free from aberrant inflammation and infection. The actions of SP-D include aggregation of pathogens, antimicrobial activity against pathogens such as *Klebsiella,* increased phagocytosis and clearance of apoptotic cells, and regulation of mediator production (20). SP-D consists of four main regions which include an N-

terminal domain, a collagenous tail, a neck region and a carbohydrate recognition domain; it exists as a trimer. Through its carbohydrate recognition domain, SP-D binds carbohydrates in a calcium-dependent manner (20, 21) and via the N-terminal region, the trimeric units oligomerise to give rise to a dodecameric cross-like structure. These can further form oligomers or 'stellate multimers', which increases the strength to bind carbohydrates and agglutinate various pathogens (20).

SP-D levels in preterm infants and evidence for recombinant fragment Surfactant protein D as a therapeutic agent

Bronchoalveolar lavage (BAL) samples taken from preterm infants over the first few days after birth have demonstrated low concentrations of SP-D in association with RDS that were associated with an increased risk of BPD (22, 23). Binding assay studies evaluating the lectin activity of SP-D demonstrate that the SP-D present in the BAL of preterm infants was less effective than that in term infants (23). Sepsis in preterm infants can be life-threatening and contributes significantly to the inflammation seen in BPD. Further, SP-D concentrations increase in preterm infants in the presence of sepsis, demonstrating its potential role as an acute phase reactant (24). Given the known interactions of SP-D to bacterial, viral and fungal pathogens (20, 25), intervention with SP-D would be expected to promote their clearance in this vulnerable population and reduce further damage. Finally, in SP-D knock-out mouse models (26), emphysematous changes are seen that are similar to those seen in the lungs of preterm infants.

Given these homeostatic and anti-inflammatory roles of SP-D, it is an attractive target for therapy, and if administered early to preterm infants there would be a reduction in inflammation by down-regulation of the pro-inflammatory signalling pathways in addition to interaction with common pathogens that induce inflammation such as *Escherichia coli. In vivo* studies using preterm lambs given recombinant full-length SP-D in addition to commercially available surfactant (which lacks SP-A and SP-D) showed a clear reduction in the pro-inflammatory cytokines such as interleukin-8 (IL-8) (27), which provides encouraging data for its potential clinical use in this population.

In practice, the properties of full-length SP-D (including varying degrees of oligomerisation, limited

solubilisation and potential aggregation at higher concentrations) make it difficult to develop a stable preparation that could be administered. Therefore, recombinant fragments of human SP-D have been explored in translational models as a potential therapy for BPD. Pre-clinical data showed the efficacy of rfhSP-D treatment in reducing and correcting inflammation in chronic inflammatory lung disease caused by SP-D deficiency. SP-D knock-out mice develop symptoms of chronic obstructive pulmonary disease (COPD) and emphysema relevant to BPD, which are correctable following treatment with recombinant SP-D (26, 28).

A stable form of rfhSP-D has been produced using a mammalian cell line and purified using affinity chromatography using a *N*-Acetylmannosamine (ManNAc)-coupled matrix as described previously (29). The recombinant fragment comprises the neck, CRD and eight gly-Xaa-Yaa repeats similar to that described for a bacterially expressed recombinant fragment of human SP-D (30). The carbohydrate recognition domain is the functional anti-inflammatory and anti-infective part of the protein without the long collagenous tail and the suggested pro-inflammatory N-terminal region (30). The rfhSP-D proposed as an investigational medicinal product (IMP) retains its anti-inflammatory properties when used as an adjunct to exogenous surfactant therapy administered via an endotracheal tube in a well-established translational model using preterm ventilated lambs (31). The endotoxin content is less than 0.05 EU/mg rfhSP-D.

Justification for the dosage regimen in the safety trial

The proposed regimen is based on the estimation of human equivalent dosages based on effective dosing in animals. In murine studies, the replacement dose of rfhSP-D was 10 micrograms daily. Assuming an average mouse weight of approximately 10-20 g, this approximates to 1 to 2 mg/kg per day. The effective dose of rfhSP-D in the preterm lamb has been estimated to be 1.5 mg/kg (unpublished data). In current practice, the administration of 100-200 mg/kg of surfactant replacement would contain 1-4 mg/kg if a naturally occurring product was used. Hence after due consideration, we elected to trial three potential dose levels of rfhSP-D, namely 1, 2 and 4 mg/kg/dose.

Study Objectives

RESPONSE is a phase I study and aims to assess the safety of 3 intratracheal dose levels (1 mg/kg/dose, 2

mg/kg/dose and 4 mg/kg/dose) of rfhSP-D in extremely preterm ventilated infants at risk of BPD.

The primary objectives are:

- To assess the safety profile of rfhSP-D across 3 dose levels based on the occurrence of dose-limiting events (DLEs) as defined below.
- To establish the Recommended Phase 2 Dose (RP2D) of rfhSP-D for preterm infants born before 28 weeks of gestation.

Secondary objectives are:

- To evaluate systemic absorption of rfhSP-D using serial measurements of SP-D in plasma and its continued presence in tracheal fluid.
- To determine the effect of rfhSP-D on inflammatory markers in lung secretions and plasma (e.g. cell counts of neutrophils, macrophages, IL-8, IL-6, IL- 1).
- To compare the clinical effects of intratracheal administration of rfhSP-D on physiological and intensive care parameters in treated infants in this trial with non-treated infants from a parallel observational cohort study of untreated infants.

Methods and Analysis

Trial design

The study will be conducted in a single-centre, tertiary level 3 neonatal intensive care unit. The study was opened on the 6th February 2024 and has a proposed 12 month recruitment period. To date the sentinel infant has been recruited to the study. This study utilises a Bayesian continual reassessment model (32-34), a model-based design that informs how the dosage of rfhSP-D should be adapted for the next participant cohort based on past trial data. For this first-in-human study, a dose escalation design will be used (Figure 1).

The three dose levels to be considered are 1mg/kg/dose, 2mg/kg/dose and 4mg/kg/dose. Participants will be enrolled at each dose level with a minimum of three participants per dose level. Each participant will

receive 3 doses of rfhSP-D at 0 hours, 12 hours and 24 hours provided that they continue to meet the inclusion criteria and are clinically stable. The first dose of rfhSP-D should be administered after standard surfactant therapy has been given. Whether or not the dose level is escalated will depend on the occurrence of DLEs in all current participants and the doses they have received. A model will be used to estimate the risk of DLE per dose level. Initial estimates of these risks will be updated using data collected throughout the trial. A one-parameter empiric model will be used to describe the relationship between the dose and the probability of observing a DLE. The CRM model will not allow dose-skipping. The target level of dose-limiting events level is set at no greater than 20%. Before the trial, the parameter of the model will be assigned a non-informative prior distribution and initial estimates of DLE probabilities will be derived using model calibration. The recommended phase two dose will be defined by considering safety and will be the highest dose level that has an estimated probability of DLE closest but below the target DLE level of no greater than 20%.

Dose escalation procedure

A schema of the dose escalation procedure and review is shown in Figure 2. The sentinel baby is the very first baby recruited to the study and this baby must be greater than 26 weeks gestational age (GA). The sentinel baby must have received all three administrations of the investigational medicinal product (IMP) and have had 72 hours of observed data post administration of 3rd administration before further infants can be recruited for the study. If the first participant does not receive all three doses of the IMP then data will still be collected but they will not qualify as the sentinel baby for this study. All infants recruited after the sentinel baby will be from 23 weeks to 27 weeks and 6 days GA for the remainder of the study. A data and safety monitoring board (DSMB) review of the neonatal adverse event severity score (NAESS) data will take place after each infant (for the first 3 participants at each dose level) has received the final dose of IMP and 72 hours of monitoring. The DSMB will evaluate the safety data before further participants can be recruited i.e the 2nd or 3rd baby cannot be recruited until data from the 1st or 2nd baby has been reviewed. This will only be for the first three infants at each dose level, thereafter the data will be reviewed in cohorts of three unless there are safety concerns. Following the recruitment of 3 infants at any dose level all safety data will be

reviewed by the DSMB and they will then advise the TSC before a decision is made to: a) move to next dose level or b) to stay at the same dose level or c) decrease the dose level or d) stop the trial. The final decision of dose escalation will be made by the TSC.

Continuous recruitment model during dose escalation decision period

The rationale for continuous recruitment in this trial is to minimise delays to recruitment during the DSMB review for overall dose escalation which takes place after a minimum of 3 participants at a dose level. It also allows for the trial of the IMP in a larger number of participants at the lower dose levels, allowing for better characterisation of the dose-response curve and the safety profile of rfhSP-D. This means that in the 1 mg/kg and 2 mg/kg cohorts, up to a further 2 participants can be recruited whilst the DSMB review dose escalation provided that no DLE has occurred in the first three participants of the dose cohort. The continued recruitment of up to two additional participants at the same or lower dose level, whilst the DSMB conduct their review, will only be permitted if there are no concerns that a DLE has occurred in the cohort under review i.e. the first 3 infants under review. Any adverse event data collected for the additional two recruited participants during DSMB review will then be reviewed by the DSMB once the 72 hour follow-up period is completed. If at any point there are concerns regarding DLEs in these additional participants, but dose escalation has occurred, then this may lead to a de-escalation. The data from the additional participants will be included at that point in the CRM which may recommend dose de-escalation in the middle of the next cohort until further data can be reviewed by the DSMB and the TSC at the next opportunity. This methodology of continual reassessment ensures that infants are only treated at the safest dose level whilst the safety profile is characterised.

Study intervention and outcomes

Eligibility criteria

All preterm infants born before 28 weeks of gestation, intubated and treated with surfactant for RDS who are considered clinically stable are eligible. Eligibility will be confirmed within 2 hours of admission to the neonatal unit and re-confirmed for each participant before the IMP is administered.

Inclusion criteria

- Inborn infants born at between 23 weeks and 0 days and 27 weeks and 6 days of gestation (<28 weeks), who are:
    - Intubated or intubation planned for RDS at the time of eligibility check within 12 hours from the time of birth.
    - Receiving standard surfactant replacement therapy.
    - Clinically stable on mechanical ventilation - clinical stability is defined at the time of IMP instillation and is defined below.
- Written informed consent from parents/guardians/person with legal responsibility has been given.

Definition of clinical stability:

Eligibility of the participant must be rechecked prior to administration of the IMP given the varying clinical status of these infants. Stability will consider if the following are true:

- Blood gas parameters within the normal range for preterm infants (pH>7.20; $paCO_2$ <8kPa).
- Mean blood pressure with or without inotropic support at a value in mmHg at least numerically equivalent gestational age in weeks or above.
- No evidence of a pneumothorax.
- Clinical observations within acceptable range for an infant of that gestational age.
- The attending neonatologist considers the infant to be clinically stable.

Exclusion Criteria

- Congenital anomalies (i.e. any major antenatal diagnosed congenital abnormality) such as congenital heart disease, suspected or known chromosomal abnormalities.
- Infants requiring only non-invasive respiratory support i.e. no endotracheal intubation
- Infants born in very poor condition and judged too sick or unstable to be included (high risk of imminent mortality) in an experimental first-in-human study; for example, infants that require maximal intensive care therapy and have findings such as a grade IV intraventricular haemorrhage that may be life-limiting.
- Infants that are born outside the participating site.

- Participation in any other interventional study (participation in another observational study is permissible).
- Parents/legal guardians are unable to give consent due to learning or other difficulties.

Recruitment and Informed consent.

The study team will monitor admissions of any women in threatened or established preterm labour. Any identified women will be approached by the study team to discuss and consider the study and verbal consent for participation will be taken. All parents/legal guardians of eligible participants will be approached if the baby is born and remains eligible for informed written consent. If the person(s) providing consent on behalf of the infant does not speak English, every effort will be made to use translational service to provide an opportunity to participate in the study. If the investigator is not able to confidently take informed consent the infant will not be recruited to the study.

Study Intervention

The recombinant fragment of surfactant protein D drug product has been manufactured to good manufacturing practice (GMP) and is known as rfhSP-D in this study. The IMP has orphan designation with the Food and Drug Administration (FDA). The sterile IMP is formulated in 0.9% saline at a concentration of 1 mg/mL in 2mL vials. The first administration of rfhSP-D will be given as soon as possible (ideally within 2 hours) after administration of standard-of-care surfactant therapy, this will be known as T0. Subsequent administration will be given at T0+12 hours and T0+24 hours. If the infant requires further standard surfactant therapy which coincides with the time of IMP administration, then the IMP should be given after the standard surfactant therapy has been administered. The IMP will be administered via a surfactant giving set that is inserted into the endotracheal tube. If the participant is extubated before any IMP dose is scheduled, then the IMP will not be administered. Vital signs will be monitored every 15 minutes for the first hour after administration of the IMP. The study drug can be administered by any authorised medically trained delegate. Eligibility criteria will be confirmed prior to each administration.

Criteria for discontinuing participation in the trial.

Any dose modifications in this protocol will be in line with the trial design and according to the dose level

confirmed by the DSMB and the TSC.

Reasons that the intervention may be discontinued are:

- Any change in the infant's condition that in the clinician's opinion justifies the discontinuation of treatment.
- Withdrawal of consent for treatment by the parent/guardian/legal representative.

Participants who discontinue protocol treatment, for any of the above reasons, will remain in the trial for follow-up and data analysis. The study team do not anticipate problems with intervention adherence given that RESPONSE is an inpatient-based study. All participants in the study will receive standard neonatal care and there will be no alteration in their clinical management. The study does not require any additional follow-up of the participants recruited once they are discharged from the hospital or reach 40 weeks postmenstrual age. The hospital where the participant is being cared for is responsible for any medical care. The sponsor holds indemnity for any trial-related harm caused to the participant.

Study Outcomes

The primary outcome of this study is to assess the safety profile of rfhSP-D across three dose levels and to identify the RP2D. DLEs will be identified using clinical criteria and grading as described below.

Dose Limiting events (DLE)

The severity of all adverse events (AE) and/or adverse reactions (AR)s (serious and non-serious) in this trial will be graded using the toxicity graded in the NAESS v1.0 (35). The NAESS has been developed as existing scores such as the Common Terminology Criteria for Adverse events (CTCAE) is not suitable for use in a study involving neonates. The NAESS developed by the International Neonatal Consortium has been developed to facilitate the conduct and appropriate interpretation of neonatal clinical trials such as RESPONSE (36).

Grades for neonatal-specific adverse events according to the NAESS v1.0 (35) are:

Grade 1: Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; no change in baseline age-appropriate behaviours*; no change in baseline care or monitoring indicated.

Grade 2: Moderate; resulting in minor changes of baseline age-appropriate behaviour*; requiring minor changes on baseline care or monitoring*+.

Grade 3: Severe; resulting in major changes of baseline age-appropriate behaviour* or non-life-threatening changes in basal physiological processes+ requiring major change in baseline care or monitoring**

Grade 4: Life-threatening; resulting in life-threatening changes in basal physiological processes+; requiring urgent major change in baseline care***.

Grade 5: Death

*Age-appropriate behaviour refers to oral feeding, voluntary movements and activity, crying pattern, social interactions and perception of pain .
+ Basal physiological processes refers to oxygenation, ventilation, tissue perfusion, metabolic stability and organ functioning
** Minor care changes constitute: brief, local, non-invasive or symptomatic treatments
*** Major care change constitute: surgery, addition of long-term treatment, and upscaling care level.

The DSMB will determine the occurrence of a DLE based on the following criteria:

- A single event defined as Grade 3 or above on the NAESS that is possibly, probably or definitely thought to be related to the IMP. Relatedness will be confirmed by an independent neonatologist at the participating site.
- A single serious adverse event (SAE) that is possibly, probably, or definitely thought to be related to the IMP. Relatedness will be confirmed by an independent neonatologist at the participating site.
- **Concerns over frequency** of any adverse events reported at grade 2 on the NAESS that are possibly, probably or definitely thought to be related to the IMP.

Secondary outcomes related to efficacy for this study are:

- Evaluation of systemic absorption of rfhSP-D using serial measurements of SP-D in plasma and its continued presence in tracheal fluid.
- To determine the effect of rfhSP-D on inflammatory markers in the lung secretions (e.g. cell counts of neutrophils, macrophages, matrix metalloproteinases, neutrophil elastase, IL-8,IL-6, IL-1).
- To compare the clinical effects of endotracheal administration of rfhSP-D on physiological and intensive care parameters in treated infants in this trial with non-treated infants from a parallel

observational study.

Participant timeline

The first administration of rfhSP-D will be given as soon as possible after administration of standard-of-care surfactant therapy, this will be known as T0. Subsequent administration will be given at T0+12 hours and T0+24 hours. Eligibility and screening investigations will be done before each administration of the IMP as shown in the schedule of events table 1.

Study Visit	Screening	Baseline	Pre Instillation of IMP T0	Pre Instillation of IMP T0 +12h	Pre Instillation of IMP T0 +24h
Time Windows	-	-	<15mins prior to instillation	<15mins prior to instillation	<15mins prior to instillation
Informed consent	*				
Eligibility	*				
Clinical stability		*	*	*	*
Demographics (incl. Gestational Age)	*	*			
Pregnancy and delivery history		*			
Stabilisation history		*			
Clinical assessment (anomalies)	*				
Vital signs	*	*		*	*
Oxygen concentration	*	*		*	*
Ventilator modality		*		*	*
Ventilator settings		*		*	*
Haematology (as per Standard of Care)		*			*
Biochemistry (as per Standard of Care)		*			*
Cytokine levels (plasma)		*			*
Cell counts GA/ETA		*		*	*
Surfactant replacement	*				
Plasma SP-D and rfhSP-D levels		*		*	*
Blood gases	*	*		*	*
SP-D levels GA		*			
rfhSP-D and SP-D level ETA				*	*
Concomitant medication		*		*	*
Cranial ultrasound scan		*			*
Review of AEs and SAEs (from time of consent)		*	*	*	*
Review of DLEs		*	*	*	*

Table 1. Schedule of events at screening and prior to administration of the IMP

Further clinical data will be collected as per the time points outlined in table 2 schedule of events

Study Visit	T0 +36h	T0 +48h	T0 +72h	T0 +96h	T0 +7d	T0+14d (±2d) T0+21d (±2d)	36w PMA	40w PMA or Hospital

	(±6h)	(±4h)	(±12h)	(±12h)	(±2d)	T0+28d (±2d)	(±1d)	Discharge (±1w)
Vital signs	*	*	*	*	*	*	*	*
Oxygen concentration	*		*	*	*	*	*	*
Ventilator modality	*	*	*	*	*	*	*	*
Ventilator settings	*	*	*	*	*	*	*	*
Haematology (SoC)		*	*	*	*			
Biochemistry (SoC)		*	*	*	*			
Plasma cytokine levels		*	*	*	*		*	
Plasma SP-D and rfhSP-D		*	*	*	*		*	
Blood gases		*	*	*	*	*	*	
ET cell counts	*	*	*	*	*			
ET rfhSP-D and SP-D levels		*	*	*	*		*	
Concomitant medication	*	*	*	*	*	*	*	*
Cranial ultrasound scan		*			*	*	*	*
Walsh Oxygen Test							*	
Review of AEs and SAEs	*	*	*	*	*	*	*	*

Table 2. Subsequent time points in participant timeline following IMP administration. SoC: as per standard of care; ET: endotracheal aspirate (if remains intubated)

The RESPONSE study will collect clinical data and biological specimens (blood, tracheal and gastric aspirates) as per table 1 and 2. Parents/guardians/those legally responsible for the participant will have the option to give consent for any anonymised data and samples that are collected as part of RESPONSE to be used in other ancillary studies that have ethical approval. Gastric secretions will be taken from all infants at the time of admission after placement of oro/naso-gastric tube and will be discarded if consent is not obtained. Blood samples (0.5mls of blood in an ethylenediaminetetraacetic acid, EDTA microtainer) will be collected at birth, 12 hours, 24 hours, 48 hours, 72 hours, 96 hours, day 7 and at 36 weeks postmenstrual age.

Data collection and management

Participants once recruited to the RESPONSE study will be allocated a study number so that all data and samples that are taken are anonymized e.g RES_001. Participation in the clinical study will be recorded in the participant clinical records. Participants will be enrolled by the study team on the OpenClinica database. Participant clinical and laboratory data will be entered directly into the password protected study database. After completion of the trial the data will be exported and retained in restricted folders by the sponsor. All data will be held for 10 years following the completion of the trial.

Primary outcome data collection in this study (safety profile of rfhSP-D) will be done through grading and

analysis of the incidence of DLEs. Potential causality of the DLE to the IMP will be assessed by an independent neonatologist. In addition to the dose-limiting events the following datasets will be collected from electronic patient records:

- At screening and on eligibility assessment: sex of participant, ethnicity, maternal medical history, antenatal steroid courses, date and time of rupture of membranes, concerns about maternal sepsis, ventilatory requirement on admission and administration of standard exogenous surfactant.

Secondary outcome data collection:

- Data will also be collected at the time points specified in the schedule of events (Tables 2 and 3) and this will include: concomitant medication, ventilatory support and parameters, known positive microbiology, use of postnatal steroids, presence and treatment of pulmonary hypertension, pneumothorax, patent ductus arteriosus, use of postnatal steroids.
- At 36 weeks PMA all participants will be assessed for severity of BPD and data will be collected about complications of prematurity such as episodes of necrotizing enterocolitis and retinopathy of prematurity. The severity of BPD in participants will be defined as per the 2019 NICHD criteria (36), an oxygen reduction test will be done if the participant is eligible (requiring less than Fi02 < 0.3 or 1.1L/min and not on positive pressure support) and remains an inpatient at the recruiting site.

All data will be handled in accordance with the Data Protection Act 2018 and GDPR and all study members will have current GCP training and certification.

Analysis of biological samples

Biological samples will be collected as per the schedule of events. Samples will be labelled with the participant's study number and transported to the Targeted Lung Immunotherapy Laboratory, UCL. Surfactant components, inflammatory markers and level of SP-D will be analysed using the ELISA technique (ELLA, BioTechne) with single marker studies and multiplex assays. Cytokines to be analysed include IL-1β, IL-6, IL-8, IL-11, IL-10, IL-13, matrix metalloproteinase-9 and tumour-necrosis factor-α. Cell counts (lymphocytes, neutrophils and macrophages) in gastric and tracheal aspirates will be assessed using flow cytometry. Samples will be retained if consent has been given by the parent/legal guardian for 5 years for

use in any other ethics-approved studies. If consent is not given for use in further studies or at the end of 5 years, the biological samples will be destroyed as per the laboratory standard operating procedure.

Sample size and Statistical analysis

As this is a safety study, no formal sample size calculation has been performed. A sample size of 24 infants is planned to meet practical recruitment and time targets and to collect sufficient data to quantify the estimated risk of DLE at each dose level. Participants with unclear safety outcomes or who have not started study treatment will be replaced to meet our planned effective sample size of 24 participants.

The primary outcome of interest is the occurrence of DLEs at the dose levels under investigation and the identification of the dose(s) that, for infants of particular risk profiles defined by gestational age, have an estimated risk of DLE closest to the target side effect level of no greater than 20%. The use of Bayesian methodology to estimate risks will allow information to be borrowed across dose levels, making the dose-escalation and RP2D identification procedure more efficient than a standard rule-based approach.

The operating characteristics of the design, for three specific scenarios, are shown in Table 3. The first scenario is one where the initial a-priori DLE probabilities calculated by calibration (halfwidth of the indifference interval set at 0.05) correspond to the true underlying probabilities of DLE. The second scenario is such that the true DLE rate of the second dose level corresponds to the target DLE rate. The third scenario is one where the true probabilities are much lower than the a-priori probabilities.

Starting dose level 1, dose-skipping not allowed, 3 dose levels, maximum 24 participants.
Skeleton (a-priori probabilities of DLE) = 0.05, 0.11, 0.20
Target DLE rate = no greater than 20%

	Dose level		
	1	**2**	**3**
Scenario 1; recommendation [%]	2.5	24.5	73.0
Scenario 2; recommendation [%]	22.5	46.5	31.0
Scenario 3; recommendation [%]	0.0	3.3	96.7

Scenario 1: true probabilities = a-priori probabilities
Scenario 2: true probabilities = 0.10, 0.20, 0.30
Scenario 3: true probabilities = 0.02, 0.05, 0.10

Table 3. Operating characteristics of the Continuous Reassessment Model

Type and grade of DLEs, SAEs and AEs will be tabulated per dose level, and further summarised by risk group

defined by gestational age. Mean estimated risk of DLE per dose level and 95% credibility intervals will be calculated using the study model. Secondary objectives will be described per dose level and risk category. Categorical variables will be summarised by frequencies and percentages, and continuous variables by means/medians and standard deviation/interquartile ranges per dose level.

Interim analyses of dose-limiting events

Interim analysis will done to assess if DLEs have occurred after each infant (for the first 3 participants at each dose level) has received the final dose of IMP and 72 hours of monitoring and all NAESS data will be reviewed by the DSMB. The DSMB will evaluate the safety data before further participants can be recruited, this will only be for the first three infants at each dose level. The purpose of this is to ensure there are no safety concerns. For the remainder of the study interim analysis will be done after cohorts of three participants are recruited at any dose level to assess the occurrence of DLE and review all clinical data. Overall DSMB review of all data to advise the TSC regarding dose escalation will take place after recruitment of 3 infants at any dose level. The trial statistician will calculate and provide updated summaries of the estimated risk of dose-limiting toxicity at each dose level. The DSMB will then advise if dose escalation can occur. There will be no interim analysis for the secondary outcomes.

The study will be terminated if any of the following stopping rules are satisfied:

- There is at least 90% chance that the risk of DLE at dose level 1 is greater than the target of 20%. If the trial is terminated under this rule, no drug dose will be recommended due to safety concerns.
- The number of participants that have been treated without side effects is deemed sufficient.
- There is evidence of increased mortality or morbidity in the participants treated with the IMP.

Study Oversight and Monitoring

The sponsor will provide trial oversight and verify the trial processes and prompting corrective action to the clinical study team as required. An independent TSC will provide oversight of the trial to safeguard the interests of the trial participants. The TSC will also provide advice to the chief investigator (CI), CCTU and the funder on all aspects of the trial through its independent chair. An independent DSMB is assigned with an allocated chair. The DSMB will be responsible for monitoring and accumulating the safety data and

making recommendations to the TSC on whether the trial should continue as planned. The DSMB will consider data as per the statistical analysis and make recommendations to the TSC chair for consideration by the TSC.

Patient and Public Involvement:

The TSC has a patient representative and the patient-facing documents have been reviewed and commented on by the patient representatives.

Adverse event reporting

All adverse events grade 3 or above on the NAESS/ SAE will be reported to CCTU within 24 hours until the participant reaches Day 7 following the last administration of the IMP (pre-clinical studies have demonstrated that the IMP is not detectable in plasma sample taken 24 hours after final administration). All related events that are graded 1 or 2 according to the NAESS will be reported within 7 days. After Day 7 any events that are considered related to the IMP will be reported within 24 hours of knowledge to the sponsor. Assessments for, and reporting of all adverse events related/unrelated will continue until 40 weeks PMA or hospital discharge. All aggregated adverse events data will be considered by the DSMB at each meeting to confirm that there are no trends, safety signals or safety concerns. Examples of adverse events that are exempt from reporting are those that are graded 1 or graded 2 according to the NAESS criteria if considered not related to the IMP. These are common observations in pre-term infants and do not require a change in clinical management unless sustained, i.e. grade 3 and above on the NAESS. There is no formal frequency of audit for this study and but will be overseen by the sponsor and if required by the Medicine and Healthcare products Regulatory Agency.

Significance of Study

Despite the medical advances in neonatal medicine, the incidence of BPD has not changed over the years, and one may argue that it has increased because we see an increasing number of extremely preterm infants survive to discharge. The life-long morbidity associated with BPD has significant implications for healthcare systems around the world.

Infants at highest risk of BPD are born at a gestational age when the majority of the alveolar and vascular development in the lungs occurs. Immaturity of the lungs means they have a developmental deficiency of surfactant leading to RDS. Ongoing lung injury secondary to postnatal insults such as infection and mechanical ventilation leads to ongoing interruption to lung development. Efforts have been made over the year to reduce these insults by changes in ventilation strategies, early nutrition, and proactive management of infection in the hope that the lungs will repair and remodelling will lead to recovery of the lung parenchyma. However, a significant number of preterm infants will have multiple insults and despite best efforts will have abnormal repair with little lung recovery leading to BPD. Inflammation remains at the centre of the pathophysiology of BPD and the most promising target for therapies. Given this, there is a need for novel anti-inflammatory therapies to be explored such as SP-D.

The role of SP-D in lung immune homeostasis is well established but due to its propensity to oligomerise does not lend itself well to a stable drug form. The proposed recombinant fragment of SP-D has been developed into a stable drug form for endotracheal administration and animal studies in a well-established translational model have demonstrated its potential anti-inflammatory effects. This phase I safety study using dose escalation of 1-4mg/kg will aim to identify a recommended Phase 2 dose for a subsequent randomised Phase 2 study in preterm infants born at less than 28 weeks gestation who are at highest risk of developing neonatal chronic lung disease.

Ethics and Dissemination

All results and analysis from the study will be published in peer-reviewed journals and presented at national and international conferences. All data generated in this study will be anonymised and the study will be conducted per Good Clinical practice. Access to the full study protocol will be given upon request and participant-level data will only be given if authorised by the sponsor for auditing purposes. Any substantial or non-substantial amendment to the study must be approved by the Health Research Authority and will be communicated with the NHS trust research and development team to ensure site implementation. Any study material that is related to participant information or informed consent will be submitted to the principal research ethics committee for approval. This study has been approved by

London-Brent NHS Research Health Authority ethics committee (REC reference 23/LO/0381) and has clinical trials approval (CTA 20363/0453/001-0001) in place.

Abbreviations

AE: Adverse Events

AR: Adverse Reaction

BAL: Bronchoalveolar Lavage

BPD: Bronchopulmonary dysplasia

CCTU: Comprehensive Clinical Trials Unit

CRM: Continual reassessment method

CTCAE: Common Terminology Criteria for Adverse Events

DLE: Dose Limiting Event

DSMB: Data and Safety Monitoring Board

GA: Gestational Age

IL: Interleukin

IMP: Investigational Medicinal Product

MTD: Maximum Tolerated Dose

NAESS: Neonatal Adverse Event Severity Score

PMA: Post Menstrual Age

rfhSP-D: Recombinant Fragment of Human Surfactant Protein D

RP2D: Recommended phase 2 dose

SAE: Serious Adverse Event

SP: Surfactant Protein

TSC: Trial Steering Committee

UCL: University College London

Acknowledgements

We are grateful to our funders, the Medical Research Council for their ongoing support with this study. We would like to thank the UCL CCTU and the UCL/UCLH Joint Research Office team for their continued support and contribution in RESPONSE trial.

Contibutors statement

RB, JM, TC, KC, HMD, NM and HC contributed to the study design and ethics application. RB, JM, and TC planned and wrote the laboratory analysis plan. HMD devised the statistical analysis plan. RB, KC, NM and HC designed and planned the clinical data collection, designed the clinical case report forms. RB, HC, JM and NM wrote and edited the clinical trial protocol. RB wrote the manuscript. RB, JM, TC,KC, HMD, NM and HC have reviewed and approved the manuscript.

Competing interests

The authors declare they have no competing interests.

Funding Statement:

This work is supported by the Medical Research Council (MRC) DPFS grant, grant number MR/P026907/1.

Data Sharing Statement

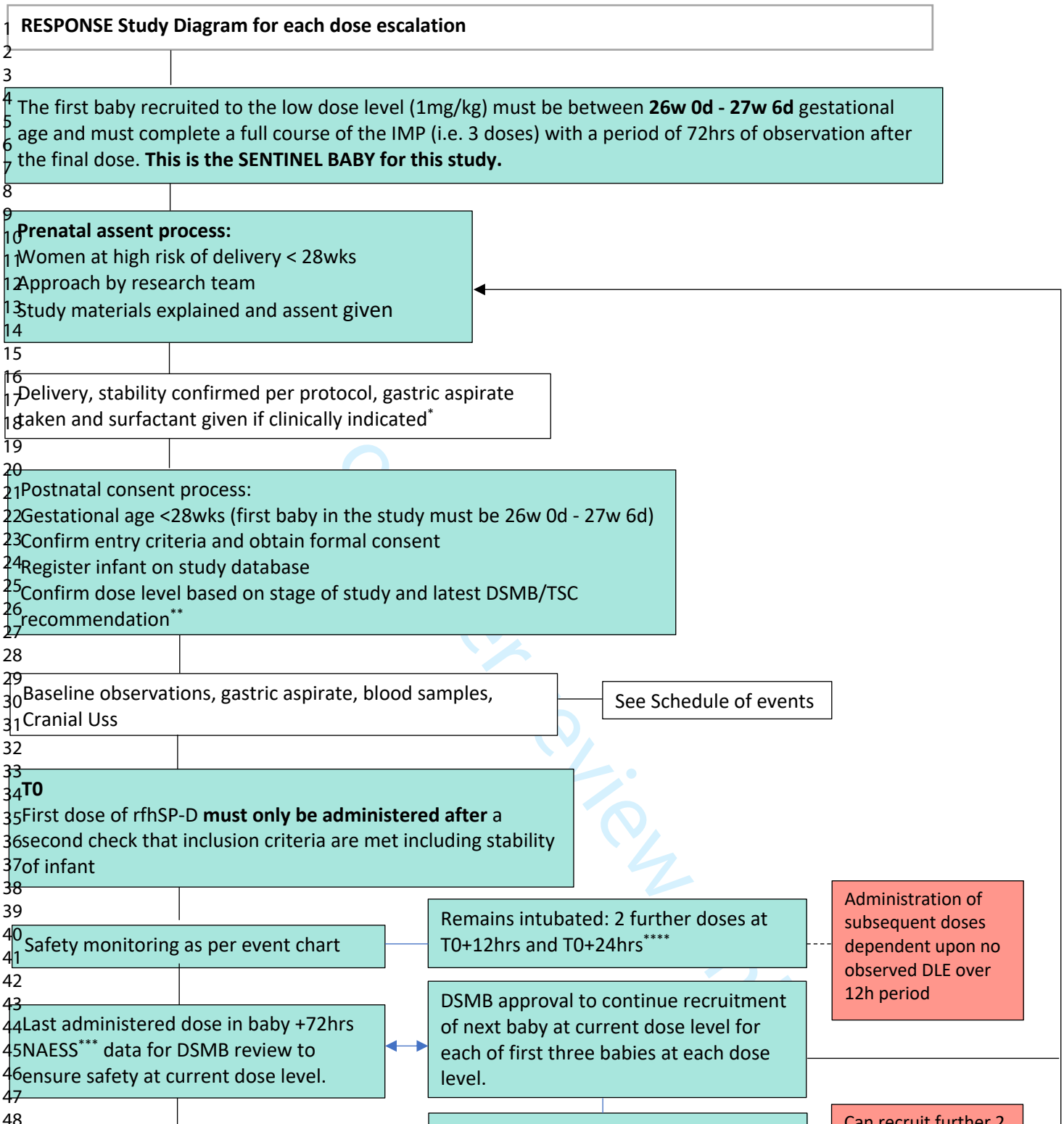
All trial data will be anonymised and entered on OpenClinica. On completion of the trial data will be available on reasonable request from the sponsor.

References

1. Abman SH, Bancalari E, Jobe A. The Evolution of Bronchopulmonary Dysplasia after 50 Years. American Journal of Respiratory and Critical Care Medicine. 2017;195(4):421-4.
2. JOBE AH, BANCALARI E. Bronchopulmonary Dysplasia. American Journal of Respiratory and Critical Care Medicine. 2001;163(7):1723-9.
3. Siffel C, Kistler KD, Lewis JFM, Sarda SP. Global incidence of bronchopulmonary dysplasia among extremely preterm infants: a systematic literature review. The Journal of Maternal-Fetal & Neonatal Medicine. 2021;34(11):1721-31.
4. Thébaud B, Goss KN, Laughon M, Whitsett JA, Abman SH, Steinhorn RH, et al. Bronchopulmonary dysplasia. Nature Reviews Disease Primers. 2019;5(1):78.
5. Bancalari E, Claure N, Sosenko IRS. Bronchopulmonary dysplasia: changes in pathogenesis, epidemiology and definition. Seminars in Neonatology. 2003;8(1):63-71.
6. Ehrenkranz RA, Walsh MC, Vohr BR, Jobe AH, Wright LL, Fanaroff AA, et al. Validation of the National Institutes of Health consensus definition of bronchopulmonary dysplasia. Pediatrics. 2005;116(6):1353-60.
7. Davidson LM, Berkelhamer SK. Bronchopulmonary Dysplasia: Chronic Lung Disease of Infancy and Long-Term Pulmonary Outcomes. Journal of Clinical Medicine. 2017;6(1):4.
8. Zysman-Colman E, Tremblay G, Bandeali S, Landry S. Bronchopulmonary dysplasia - Trends over three decades. Paediatrics & child health. 2013;18:86-90.
9. Stoll BJ, Hansen NI, Bell EF, Walsh MC, Carlo WA, Shankaran S, et al. Trends in Care Practices, Morbidity, and Mortality of Extremely Preterm Neonates, 1993-2012. Jama. 2015;314(10):1039-51.
10. Costeloe KL, Hennessy EM, Haider S, Stacey F, Marlow N, Draper ES. Short term outcomes after extreme preterm birth in England: comparison of two birth cohorts in 1995 and 2006 (the EPICure studies). BMJ : British Medical Journal. 2012;345:e7976.
11. Hurst JR, Beckmann J, Ni Y, Bolton CE, McEniery CM, Cockcroft JR, et al. Respiratory and

Cardiovascular Outcomes in Survivors of Extremely Preterm Birth at 19 Years. American Journal of Respiratory and Critical Care Medicine. 2020;202(3):422-32.
12. Um-Bergström P, Hallberg J, Pourbazargan M, Berggren-Broström E, Ferrara G, Eriksson MJ, et al. Pulmonary outcomes in adults with a history of Bronchopulmonary Dysplasia differ from patients with asthma. Respiratory Research. 2019;20(1):102.
13. Marlow N, Johnson S, Hurst JR. The extremely preterm young adult - State of the art. Semin Fetal Neonatal Med. 2022;27(3):101365.
14. Taghizadeh A, Reynolds E. Pathogenesis of bronchopulmonary dysplasia following hyaline membrane disease. The American journal of pathology. 1976;82(2):241.
15. Jobe AJ. The New BPD: An Arrest of Lung Development. Pediatric Research. 1999;46(6):641-.
16. Schultz C, Temming P, Bucsky P, Göpel W, Strunk T, Härtel C. Immature anti-inflammatory response in neonates. Clinical and experimental immunology. 2004;135(1):130-6.
17. Sullivan G, Galdi P, Borbye-Lorenzen N, Stoye DQ, Lamb GJ, Evans MJ, et al. Preterm Birth Is Associated With Immune Dysregulation Which Persists in Infants Exposed to Histologic Chorioamnionitis. Frontiers in Immunology. 2021;12.
18. Speer CP. Inflammation and bronchopulmonary dysplasia: A continuing story. Seminars in Fetal and Neonatal Medicine. 2006;11(5):354-62.
19. Possmayer F, Yu SH, Weber JM, Harding PG. Pulmonary surfactant. Can J Biochem Cell Biol. 1984;62(11):1121-33.
20. Watson A, Madsen J, Clark HW. SP-A and SP-D: Dual Functioning Immune Molecules With Antiviral and Immunomodulatory Properties. Frontiers in Immunology. 2021;11.
21. Wright JR. Immunoregulatory functions of surfactant proteins. Nature Reviews Immunology. 2005;5(1):58-68.
22. Beresford MW, Shaw NJ. Bronchoalveolar lavage surfactant protein a, B, and d concentrations in preterm infants ventilated for respiratory distress syndrome receiving natural and synthetic surfactants. Pediatr Res. 2003;53(4):663-70.
23. Kotecha S, Davies PL, Clark HW, McGreal EP. Increased prevalence of low oligomeric state surfactant protein D with restricted lectin activity in bronchoalveolar lavage fluid from preterm infants. Thorax. 2013;68(5):460-7.
24. Mackay R-MA, Townsend JP, Calvert J, Anthony M, Wilkinson AR, Postle AD, et al. Increased surfactant protein-D levels in the airways of preterm neonates with sepsis indicated responses to infectious challenges. Acta Paediatrica. 2019;108(5):870-6.
25. Clark H, Reid K. The potential of recombinant surfactant protein D therapy to reduce inflammation in neonatal chronic lung disease, cystic fibrosis, and emphysema. Archives of Disease in Childhood. 2003;88(11):981-4.
26. Clark H, Palaniyar N, Strong P, Edmondson J, Hawgood S, Reid KB. Surfactant protein D reduces alveolar macrophage apoptosis in vivo. J Immunol. 2002;169(6):2892-9.
27. Sato A, Whitsett JA, Scheule RK, Ikegami M. Surfactant Protein-D Inhibits Lung Inflammation Caused by Ventilation in Premature Newborn Lambs. American Journal of Respiratory and Critical Care Medicine. 2010;181(10):1098-105.
28. Knudsen L, Ochs M, Mackay R, Townsend P, Deb R, Mühlfeld C, et al. Truncated recombinant human SP-D attenuates emphysema and type II cell changes in SP-D deficient mice. Respir Res. 2007;8(1):70.
29. Sorensen GL, Hoegh SV, Leth-Larsen R, Thomsen TH, Floridon C, Smith K, et al. Multimeric and trimeric subunit SP-D are interconvertible structures with distinct ligand interaction. Mol Immunol. 2009;46(15):3060-9.
30. Strong P, Reid KBM, Clark H. Intranasal delivery of a truncated recombinant human SP-D is effective at down-regulating allergic hypersensitivity in mice sensitized to allergens of Aspergillus fumigatus. Clinical and experimental immunology. 2002;130(1):19-24.
31. Various A. Selected Abstracts of the 12th International Congress of UENPS; Krakow (Poland); September 2nd-4th, 2022. Journal of Pediatric and Neonatal Individualized Medicine (JPNIM). 2022;12(1):e120104.

32. O'Quigley J, Pepe M, Fisher L. Continual reassessment method: a practical design for phase 1 clinical trials in cancer. Biometrics. 1990;46(1):33-48.
33. Faries D. Practical modifications of the continual reassessment method for phase i cancer clinical trials. Journal of Biopharmaceutical Statistics. 1994;4(2):147-64.
34. Goodman SN, Zahurak ML, Piantadosi S. Some practical improvements in the continual reassessment method for phase I studies. Stat Med. 1995;14(11):1149-61.
35. Salaets T, Turner MA, Short M, Ward RM, Hokuto I, Ariagno RL, et al. Development of a neonatal adverse event severity scale through a Delphi consensus approach. Arch Dis Child. 2019;104(12):1167-73.
36. Jensen EA, Dysart K, Gantz MG, McDonald S, Bamat NA, Keszler M, et al. The Diagnosis of Bronchopulmonary Dysplasia in Very Preterm Infants. An Evidence-based Approach. American Journal of Respiratory and Critical Care Medicine. 2019;200(6):751-9.



*via Endotracheal tube only

**First iteration will be at 1mg/kg/dose, subsequent iterations 2mg/kg/dose and 4mg/Kg/dose

***Neonatal Adverse Event Severity Score (NAESS)

****administration window of +2hrs

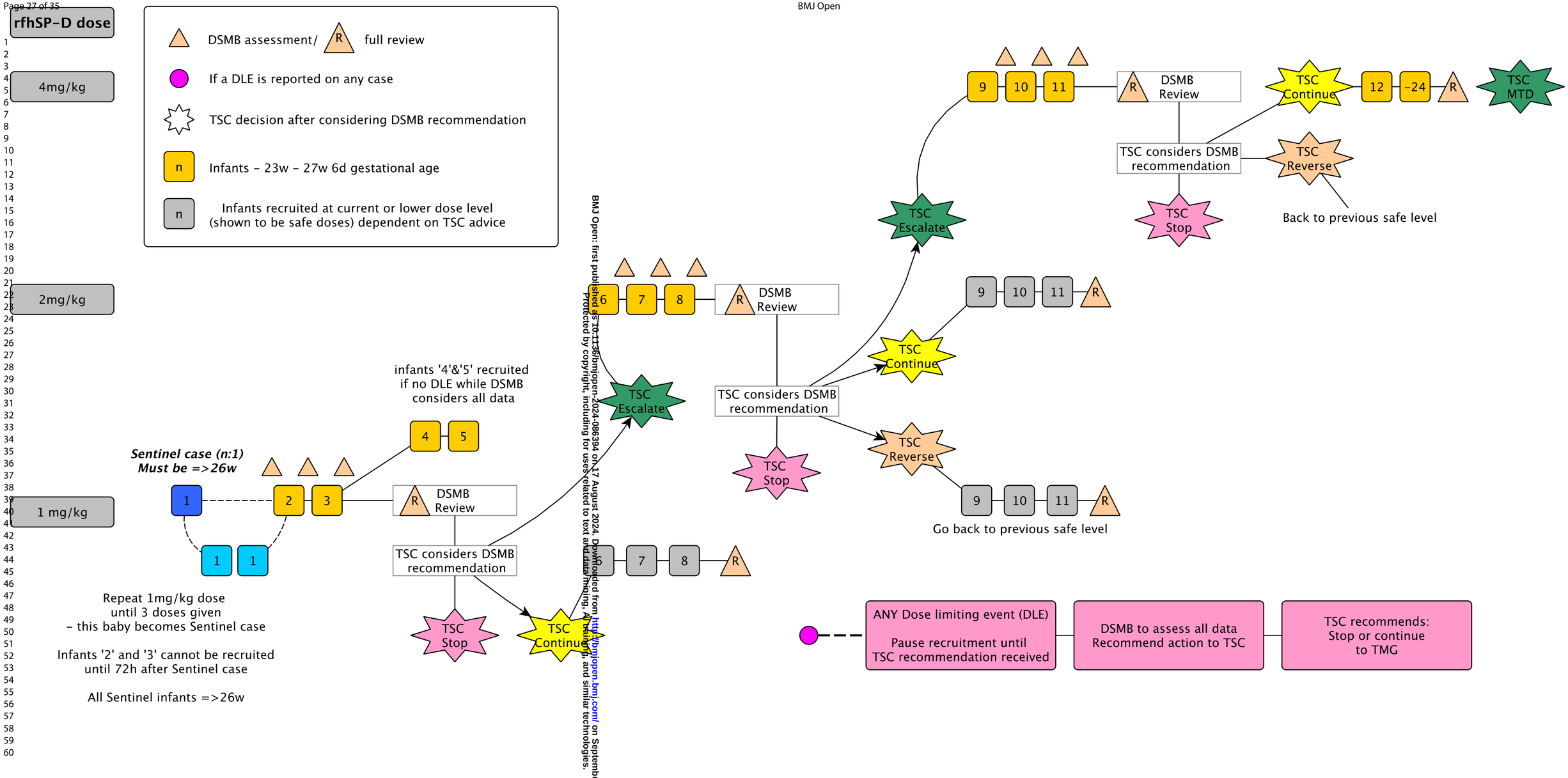


Figure 12: Schematic example of the dose escalation design using the Continual Reassessment Method. The salmon triangle refers to data review by the DSMB. which takes place
1) for the first three babies 72h after study entry after each baby at each dose level (and confirms no DLE to the management group – termed DSMB assessment),
2) after any potential DLE has been observed (and recommends trial action to TSC), and
3) in groups of three babies to make recommendation to TSC about dose escalation. In the case of 2 or 3 a full data review by the DSMB is undertaken (identified with a 'R").
In the event of a suspected DLE, the DSMB will be consulted to recommend to the TSC to continue at the same dose/reduce dose and continue or trial needs to be terminated.
If a DLE occurs, then the DSMB may recommend recruitment at the same dose level (e.g. 'grey" participants) or they may advise dose de-escalation i.e. 2mg/kg to 1mg/kg for a further 3 participants before further review, after which if no DLE occurs they may advise escalation in dose again. In the absence of DLEs the trial will continue to recruit to the same dose level during DSMB /TSC review periods.

Reporting checklist for protocol of a clinical trial.

Based on the SPIRIT guidelines.

Instructions to authors

Complete this checklist by entering the page numbers from your manuscript where readers will find each of the items listed below.

Your article may not currently address all the items on the checklist. Please modify your text to include the missing information. If you are certain that an item does not apply, please write "n/a" and provide a short explanation.

Upload your completed checklist as an extra file when you submit to a journal.

In your methods section, say that you used the SPIRITreporting guidelines, and cite them as:

Chan A-W, Tetzlaff JM, Gøtzsche PC, Altman DG, Mann H, Berlin J, Dickersin K, Hróbjartsson A, Schulz KF, Parulekar WR, Krleža-Jerić K, Laupacis A, Moher D. SPIRIT 2013 Explanation and Elaboration: Guidance for protocols of clinical trials. BMJ. 2013;346:e7586

		Reporting Item	Page Number
Administrative information			
Title	#1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	#2a	Trial identifier and registry name. If not yet registered, name of intended registry	3
Trial registration: data set	#2b	All items from the World Health Organization Trial Registration Data Set	3
Protocol version	#3	Date and version identifier	3
Funding	#4	Sources and types of financial, material, and other support	3
Roles and responsibilities: contributorship	#5a	Names, affiliations, and roles of protocol contributors	1 and 28

Roles and responsibilities: sponsor contact information	#5b	Name and contact information for the trial sponsor	3
Roles and responsibilities: sponsor and funder	#5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	3
Roles and responsibilities: committees	#5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	22
Introduction			
Background and rationale	#6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	4
Background and rationale: choice of comparators	#6b	Explanation for choice of comparators	N/A this is a phase I study and there is no control or comparator.
Objectives	#7	Specific objectives or hypotheses	8
Trial design	#8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, non-inferiority, exploratory)	8
Methods: Participants, interventions, and outcomes			

Study setting	#9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	8
Eligibility criteria	#10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	12
Interventions: description	#11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	13
Interventions: modifications	#11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving / worsening disease)	14
Interventions: adherance	#11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return; laboratory tests)	14
Interventions: concomitant care	#11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	13/14
Outcomes	#12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	14
Participant timeline	#13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	16
Sample size	#14	Estimated number of participants needed to achieve study objectives and how it was	20

		determined, including clinical and statistical assumptions supporting any sample size calculations	
Recruitment	#15	Strategies for achieving adequate participant enrolment to reach target sample size	13
Methods: Assignment of interventions (for controlled trials)			
Allocation: sequence generation	#16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	n/a - this is an open-labelled safety trial. All participants will be anonymised with a study number that will be sequential as they are recruited to the study.
Allocation concealment mechanism	#16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	n/a – this is not a blinded study but a safety phase I study.
Allocation: implementation	#16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	18
Blinding (masking)	#17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	n/a - this is a Phase I safety study with only one intervention group and there will be no blinding of the intervention.
Blinding (masking): emergency unblinding	#17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	n/a - this is a Phase I safety study.

Methods: Data collection, management, and analysis			
Data collection plan	#18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	18
Data collection plan: retention	#18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	20
Data management	#19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	18
Statistics: outcomes	#20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	20
Statistics: additional analyses	#20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	n/a - there is only one group in this protocol and therefore no subgroup analyses will be done.
Statistics: analysis population and missing data	#20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	20

Methods: Monitoring			
Data monitoring: formal committee	#21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	22
Data monitoring: interim analysis	#21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	21
Harms	#22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	22
Auditing	#23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	22
Ethics and dissemination			
Research ethics approval	#24	Plans for seeking research ethics committee / institutional review board (REC / IRB) approval	23
Protocol amendments	#25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC / IRBs, trial participants, trial registries, journals, regulators)	23
Consent or assent	#26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	13

Consent or assent: ancillary studies	#26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	16
Confidentiality	#27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	19
Declaration of interests	#28	Financial and other competing interests for principal investigators for the overall trial and each study site	25
Data access	#29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	19
Ancillary and post trial care	#30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	15
Dissemination policy: trial results	#31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	23
Dissemination policy: authorship	#31b	Authorship eligibility guidelines and any intended use of professional writers	23
Dissemination policy: reproducible research	#31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	23
Appendices			
Informed consent materials	#32	Model consent form and other related documentation given to participants and authorised surrogates	Appendix 1
Biological specimens	#33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or	20

molecular analysis in the current trial and for future use in ancillary studies, if applicable

BMJ Open

Recombinant Surfactant protein D to prevent neonatal chronic lung disease (RESPONSE): a protocol for a phase I safety trial in a tertiary neonatal unit.

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2024-086394.R2
Article Type:	Protocol
Date Submitted by the Author:	09-Jul-2024
Complete List of Authors:	Bhatt, Reena; University College London, Neonatal Intensive Care Unit; University College London, Neonatology Madsen, Jens; University College London, Department of Neonatology Castillo-Hernandez, Tania; University College London, Department of Neonatology Chant, Kathy; University College London, Department of Neonatology Dehbi, Hakim-Moulay; University College London Institute of Clinical Trials and Methodology Marlow, Neil; UCL Clark, Howard; University College London Hospitals; University College London, Department of Neonatology
Primary Subject Heading:	Paediatrics
Secondary Subject Heading:	Respiratory medicine, Research methods
Keywords:	IMMUNOLOGY, Neonatal intensive & critical care < INTENSIVE & CRITICAL CARE, RESPIRATORY MEDICINE (see Thoracic Medicine)

Title

Recombinant Surfactant protein D to prevent neonatal chronic lung disease (RESPONSE): a protocol for a phase I safety trial in a tertiary neonatal unit.

Names protocol contributors

Reena Bhatt[1,2], Jens Madsen[2], Tania Castillo-Hernandez[2], Kathy Chant[2], Hakim-Moulay Dehbi[3], Neil Marlow[2], Howard Clark[1,2]

[1] Neonatal Intensive Care Unit, EGA Wing, University College London Hospitals, London, United Kingdom

[2] Department of Neonatology, EGA Institute for Women's Health, University College London, London, United Kingdom

[3] Comprehensive Clinical Trials Unit, University College London, London, United Kingdom

Corresponding author

Dr Reena Bhatt

EGA Institute for Women's Health

Rockefeller Building

University College London

21 University Street

London, WC1E 6DE

United Kingdom

Tel: 02076796031

Email: reena.bhatt@ucl.ac.uk

Abstract

Introduction: Chronic respiratory morbidity from bronchopulmonary dysplasia (BPD) remains the most common complication of preterm birth and has consequences for later respiratory, cardiovascular and neurodevelopmental outcomes. The early phases of respiratory illness are characterised by rapid consumption of endogenous surfactant and slow replenishment. Exogenous surfactant is routinely administered to infants born before 28 weeks of gestation as prophylaxis.

Endogenous surfactant includes four proteins, known as surfactant proteins (SP) A, B, C and D. Current bovine- and porcine-derived surfactant preparations only contain surfactant proteins B and C. SP-D has a key role in lung immune homeostasis as part of the innate immune system. Laboratory studies using recombinant SP-D have demonstrated reduced inflammation, which may be a pathway to reducing the associated morbidity from BPD. RESPONSE utilises a recombinant fragment of human surfactant protein D (rfhSP-D), in a phase I safety and dose-escalation trial as the first stage in determining its effect in humans.

Methods and Analysis: This is a single centre, dose-escalation, phase I safety study aiming to recruit 24 infants born before 28 weeks gestation with respiratory distress syndrome (RDS). In addition to routine surfactant replacement therapy, participants will receive three doses of rfhSP-D via endotracheal route at either 1mg/kg, 2mg/kg or 4mg/kg. The study utilises a Bayesian Continual Reassessment Method (CRM) to make dose escalation decisions. Dose-limiting events (DLE) in this trial will be graded according to the published neonatal adverse event severity score (NAESS). The primary outcome of this study is to evaluate the safety profile of rfhSP-D across each dose level based on the profile of DLE to establish the recommended phase 2 dose (RP2D) of rfhSP-D.

Ethics and Dissemination: The RESPONSE study has received ethical approval. Results from the study will be published in peer-reviewed journals and presented at national and international conferences.

Trial registration: Medical EudraCT: 2021-001824-16, ISRCTN: 17083028, Clinical trials.gov.uk: NCT05898633.

Protocol Version: RESPONSE Protocol v3.0 25/01/2024

Name and contact information for the trial sponsor: University College London (UCL) with sponsor responsibilities delegated to the Comprehensive Clinical Trails Unit (CCTU). Contact:

cctu.response@ucl.ac.uk

Role of sponsor: Specific functions have been delegated to the UCL CCTU by the sponsor. A clinical project manager at the UCL CCTU will oversee the clinical trial manager who will be responsible for the day-to-day management of the trial. The CCTU staff will be involved in site initiation, database construction, development of the protocol and trial-related documentation. The sponsor will be responsible for the audit of the trial.

Key Words: Neonates, bronchopulmonary dysplasia, surfactant protein D, prematurity, Bayesian method

Article Summary:

Strengths:

- This study uses the International Neonatal Consortium (INC) Neonatal Adverse Event Severity Score (NAESS) which is specific to this population and allows a better grading and understanding of the adverse events and progression of the trial. This scoring system unlike others e.g. Common Terminology Criteria for Adverse events (CTCAE) takes into account age-appropriate behaviour e.g. feeding and physiological parameters such as changes in oxygenation. Although the NAESS has not been rigorously validated, it is well-placed to improve the quality of drug evaluation in this highly vulnerable population.
- This is a safety study aiming to establish a recommended phase II dose of a novel therapy in a highly vulnerable population affected by Bronchopulmonary dysplasia which has a significant impact on long-term lung health.
- This study utilises Bayesian analysis which utilises prior cohort data to inform the ongoing dose escalation.

Limitations:

- This is a single-centre study which may affect recruitment and the population characteristics.

Introduction

Clinical Need for Study

The introduction of exogenous surfactant replacement therapy has significantly improved mortality in extremely preterm infants, those born before 28 weeks of gestation (1). Despite this, chronic respiratory morbidity from BPD remains the most common complication of very preterm birth. BPD may be formally defined by the persisting need for respiratory support past 36 weeks postmenstrual age (PMA) (2). It affects up to 75% of extremely preterm infants (3), with decreasing prevalence with increasing gestational age (4). The pathogenesis of BPD is complex and multifactorial, involving lung immaturity, infection, inflammation, oxygen toxicity and ventilator-induced injury.

Unlike when first described, BPD is now rarely seen in infants born at more than 1200 g or after 30 weeks of gestation (2, 5, 6) due to the introduction of antenatal steroid administration, surfactant replacement therapy, improved ventilation strategies and better nutrition (7, 8). The prevalence of BPD has not fallen as expected (8-10). It can be argued that with advances in neonatal care leading to increased survival of infants at greatest risk of BPD, the prevalence may increase in years to come presenting a challenge for healthcare systems worldwide. Furthermore, BPD has lifelong consequences, with respiratory impairment that has important implications for adult clinicians, tracking through to adult life (11, 12) and neonatal BPD is also a marker for adult cognitive, educational and behavioural impairment with implications for health, wealth and relationships for life (13).

As the mean gestational age of neonatal populations has fallen with increasing survival, the pathophysiology of chronic respiratory disease in very preterm populations has changed. Whereas the original descriptions of BPD related the occurrence and progression primarily to barotrauma from mechanical positive pressure ventilation (14), with increasing immaturity the profile of causation has changed, and this “new” BPD (15) is primarily found among extremely preterm infants. The primary driver in its development is lung inflammation, subject to the other risks referred to above. The disease is characterised by developmental

arrest of lung tissue and a loss of alveolar septation by impairing alveolar crest development. This interruption in normal lung development with superimposed inflammation, oxygen toxicity and pressure-induced changes (barotrauma, volutrauma, atelectotrauma) completes the clinical picture.

Postnatal pulmonary inflammation is due to an imbalance in humoral factors favouring a pro-inflammatory response (16, 17) and increased presence of inflammatory cells in the airway (18). Inflammation, secondary to positive pressure ventilation, oxygen therapy or infection, may have further impact on the cytokine profile and the interruption of lung development. The overwhelming evidence for inflammation as a causal mechanism in the development of BPD suggests that early anti-inflammatory therapies might reduce the frequency and severity of the condition. Identification of potential therapeutic targets remains a goal to reduce the frequency of BPD in high-risk infants. Naturally occurring SP-D has gained increasing interest as a potential immunotherapy to dampen the pro-inflammatory cascade and facilitate lung repair, thus reducing the frequency and severity of lung disease. In turn, this may have important long-term benefits for the child.

Surfactant Protein D

Mammalian surfactant comprises largely phospholipids (80%), neutral lipids (10%) and surfactant proteins (10%), dipalmitoylphosphatidylcholine (DPPC) being the primary surface-active component at the alveolar surface (19). Four SP are found in surfactant, SP-A, SP-B, SP-C and SP-D. SP-B and SP-C are hydrophobic and their role is largely to stabilise the lipid monolayer formed at the air-liquid interface by stimulating phospholipid adsorption and reducing surface tension. Due to their hydrophobic nature, these SP are easily extracted from bovine or porcine sources and present in widely used commercial surfactants. In contrast, SP-A and SP-D are hydrophilic and are not present in the surfactant preparations currently used in clinical practice.

SP-D is an essential lung component and functions to keep the lungs in a hypo-responsive state at rest, free from aberrant inflammation and infection. The actions of SP-D include aggregation of pathogens, antimicrobial activity against pathogens such as *Klebsiella,* increased phagocytosis and clearance of apoptotic cells, and regulation of mediator production (20). SP-D consists of four main regions which include an N-

terminal domain, a collagenous tail, a neck region and a carbohydrate recognition domain; it exists as a trimer. Through its carbohydrate recognition domain, SP-D binds carbohydrates in a calcium-dependent manner (20, 21) and via the N-terminal region, the trimeric units oligomerise to give rise to a dodecameric cross-like structure. These can further form oligomers or 'stellate multimers', which increases the strength to bind carbohydrates and agglutinate various pathogens (20).

SP-D levels in preterm infants and evidence for recombinant fragment Surfactant protein D as a therapeutic agent

Bronchoalveolar lavage (BAL) samples taken from preterm infants over the first few days after birth have demonstrated low concentrations of SP-D in association with RDS that were associated with an increased risk of BPD (22, 23). Binding assay studies evaluating the lectin activity of SP-D demonstrate that the SP-D present in the BAL of preterm infants was less effective than that in term infants (23). Sepsis in preterm infants can be life-threatening and contributes significantly to the inflammation seen in BPD. Further, SP-D concentrations increase in preterm infants in the presence of sepsis, demonstrating its potential role as an acute phase reactant (24). Given the known interactions of SP-D to bacterial, viral and fungal pathogens (20, 25), intervention with SP-D would be expected to promote their clearance in this vulnerable population and reduce further damage. Finally, in SP-D knock-out mouse models (26), emphysematous changes are seen that are similar to those seen in the lungs of preterm infants.

Given these homeostatic and anti-inflammatory roles of SP-D, it is an attractive target for therapy, and if administered early to preterm infants there would be a reduction in inflammation by down-regulation of the pro-inflammatory signalling pathways in addition to interaction with common pathogens that induce inflammation such as *Escherichia coli. In vivo* studies using preterm lambs given recombinant full-length SP-D in addition to commercially available surfactant (which lacks SP-A and SP-D) showed a clear reduction in the pro-inflammatory cytokines such as interleukin-8 (IL-8) (27), which provides encouraging data for its potential clinical use in this population.

In practice, the properties of full-length SP-D (including varying degrees of oligomerisation, limited

solubilisation and potential aggregation at higher concentrations) make it difficult to develop a stable preparation that could be administered. Therefore, recombinant fragments of human SP-D have been explored in translational models as a potential therapy for BPD. Pre-clinical data showed the efficacy of rfhSP-D treatment in reducing and correcting inflammation in chronic inflammatory lung disease caused by SP-D deficiency. SP-D knock-out mice develop symptoms of chronic obstructive pulmonary disease (COPD) and emphysema relevant to BPD, which are correctable following treatment with recombinant SP-D (26, 28).

A stable form of rfhSP-D has been produced using a mammalian cell line and purified using affinity chromatography using a *N*-Acetylmannosamine **(**ManNAc)-coupled matrix as described previously (29). The recombinant fragment comprises the neck, CRD and eight gly-Xaa-Yaa repeats similar to that described for a bacterially expressed recombinant fragment of human SP-D (30). The carbohydrate recognition domain is the functional anti-inflammatory and anti-infective part of the protein without the long collagenous tail and the suggested pro-inflammatory N-terminal region (30). The rfhSP-D proposed as an investigational medicinal product (IMP) retains its anti-inflammatory properties when used as an adjunct to exogenous surfactant therapy administered via an endotracheal tube in a well-established translational model using preterm ventilated lambs (31). The endotoxin content is less than 0.05 EU/mg rfhSP-D.

Justification for the dosage regimen in the safety trial

The proposed regimen is based on the estimation of human equivalent dosages based on effective dosing in animals. In murine studies, the replacement dose of rfhSP-D was 10 micrograms daily. Assuming an average mouse weight of approximately 10-20 g, this approximates to 1 to 2 mg/kg per day. The effective dose of rfhSP-D in the preterm lamb has been estimated to be 1.5 mg/kg (unpublished data). In current practice, the administration of 100-200 mg/kg of surfactant replacement would contain 1-4 mg/kg if a naturally occurring product was used. Hence after due consideration, we elected to trial three potential dose levels of rfhSP-D, namely 1, 2 and 4 mg/kg/dose.

Study Objectives

RESPONSE is a phase I study and aims to assess the safety of 3 intratracheal dose levels (1 mg/kg/dose, 2

mg/kg/dose and 4 mg/kg/dose) of rfhSP-D in extremely preterm ventilated infants at risk of BPD.

The primary objectives are:

- To assess the safety profile of rfhSP-D across 3 dose levels based on the occurrence of dose-limiting events (DLEs) as defined below.
- To establish the Recommended Phase 2 Dose (RP2D) of rfhSP-D for preterm infants born before 28 weeks of gestation.

Secondary objectives are:

- To evaluate systemic absorption of rfhSP-D using serial measurements of SP-D in plasma and its continued presence in tracheal fluid.
- To determine the effect of rfhSP-D on inflammatory markers in lung secretions and plasma (e.g. cell counts of neutrophils, macrophages, IL-8, IL-6, IL- 1).
- To compare the clinical effects of intratracheal administration of rfhSP-D on physiological and intensive care parameters in treated infants in this trial with non-treated infants from a parallel observational cohort study of untreated infants.

Methods and Analysis

Trial design

The study will be conducted in a single-centre, tertiary level 3 neonatal intensive care unit. The study was opened on the 6th February 2024 and has a proposed 12 month recruitment period. To date the 4 infants have been recruited to the study. This study utilises a Bayesian continual reassessment model (32-34), a model-based design that informs how the dosage of rfhSP-D should be adapted for the next participant cohort based on past trial data. For this first-in-human study, a dose escalation design will be used (Figure 1).

The three dose levels to be considered are 1mg/kg/dose, 2mg/kg/dose and 4mg/kg/dose. Participants will be enrolled at each dose level with a minimum of three participants per dose level. Each participant will

receive 3 doses of rfhSP-D at 0 hours, 12 hours and 24 hours provided that they continue to meet the inclusion criteria and are clinically stable. The first dose of rfhSP-D should be administered after standard surfactant therapy has been given. Whether or not the dose level is escalated will depend on the occurrence of DLEs in all current participants and the doses they have received. A model will be used to estimate the risk of DLE per dose level. Initial estimates of these risks will be updated using data collected throughout the trial. A one-parameter empiric model will be used to describe the relationship between the dose and the probability of observing a DLE. The CRM model will not allow dose-skipping. The target level of dose-limiting events level is set at no greater than 20%. Before the trial, the parameter of the model will be assigned a non-informative prior distribution and initial estimates of DLE probabilities will be derived using model calibration. The recommended phase two dose will be defined by considering safety and will be the highest dose level that has an estimated probability of DLE closest but below the target DLE level of no greater than 20%.

Dose escalation procedure

A schema of the dose escalation procedure and review is shown in Figure 2. The sentinel baby is the very first baby recruited to the study and this baby must be greater than 26 weeks gestational age (GA). The sentinel baby must have received all three administrations of the investigational medicinal product (IMP) and have had 72 hours of observed data post administration of 3rd administration before further infants can be recruited for the study. If the first participant does not receive all three doses of the IMP then data will still be collected but they will not qualify as the sentinel baby for this study. All infants recruited after the sentinel baby will be from 23 weeks to 27 weeks and 6 days GA for the remainder of the study. A data and safety monitoring board (DSMB) review of the neonatal adverse event severity score (NAESS) data will take place after each infant (for the first 3 participants at each dose level) has received the final dose of IMP and 72 hours of monitoring. The DSMB will evaluate the safety data before further participants can be recruited i.e the 2nd or 3rd baby cannot be recruited until data from the 1st or 2nd baby has been reviewed. This will only be for the first three infants at each dose level, thereafter the data will be reviewed in cohorts of three unless there are safety concerns. Following the recruitment of 3 infants at any dose level all safety data will be

reviewed by the DSMB and they will then advise the TSC before a decision is made to: a) move to next dose level or b) to stay at the same dose level or c) decrease the dose level or d) stop the trial. The final decision of dose escalation will be made by the TSC.

Continuous recruitment model during dose escalation decision period

The rationale for continuous recruitment in this trial is to minimise delays to recruitment during the DSMB review for overall dose escalation which takes place after a minimum of 3 participants at a dose level. It also allows for the trial of the IMP in a larger number of participants at the lower dose levels, allowing for better characterisation of the dose-response curve and the safety profile of rfhSP-D. This means that in the 1 mg/kg and 2 mg/kg cohorts, up to a further 2 participants can be recruited whilst the DSMB review dose escalation provided that no DLE has occurred in the first three participants of the dose cohort. The continued recruitment of up to two additional participants at the same or lower dose level, whilst the DSMB conduct their review, will only be permitted if there are no concerns that a DLE has occurred in the cohort under review i.e. the first 3 infants under review. Any adverse event data collected for the additional two recruited participants during DSMB review will then be reviewed by the DSMB once the 72 hour follow-up period is completed. If at any point there are concerns regarding DLEs in these additional participants, but dose escalation has occurred, then this may lead to a de-escalation. The data from the additional participants will be included at that point in the CRM which may recommend dose de-escalation in the middle of the next cohort until further data can be reviewed by the DSMB and the TSC at the next opportunity. This methodology of continual reassessment ensures that infants are only treated at the safest dose level whilst the safety profile is characterised.

Study intervention and outcomes

Eligibility criteria

All preterm infants born before 28 weeks of gestation, intubated and treated with surfactant for RDS who are considered clinically stable are eligible. Eligibility will be confirmed within 2 hours of admission to the neonatal unit and re-confirmed for each participant before the IMP is administered.

Inclusion criteria

- Inborn infants born at between 23 weeks and 0 days and 27 weeks and 6 days of gestation (<28 weeks), who are:
    - Intubated or intubation planned for RDS at the time of eligibility check within 12 hours from the time of birth.
    - Receiving standard surfactant replacement therapy.
    - Clinically stable on mechanical ventilation - clinical stability is defined at the time of IMP instillation and is defined below.
- Written informed consent from parents/guardians/person with legal responsibility has been given.

Definition of clinical stability:

Eligibility of the participant must be rechecked prior to administration of the IMP given the varying clinical status of these infants. Stability will consider if the following are true:

- Blood gas parameters within the normal range for preterm infants (pH>7.20; $paCO_2$ <8kPa).
- Mean blood pressure with or without inotropic support at a value in mmHg at least numerically equivalent gestational age in weeks or above.
- No evidence of a pneumothorax.
- Clinical observations within acceptable range for an infant of that gestational age.
- The attending neonatologist considers the infant to be clinically stable.

Exclusion Criteria

- Congenital anomalies (i.e. any major antenatal diagnosed congenital abnormality) such as congenital heart disease, suspected or known chromosomal abnormalities.
- Infants requiring only non-invasive respiratory support i.e. no endotracheal intubation
- Infants born in very poor condition and judged too sick or unstable to be included (high risk of imminent mortality) in an experimental first-in-human study; for example, infants that require maximal intensive care therapy and have findings such as a grade IV intraventricular haemorrhage that may be life-limiting.
- Infants that are born outside the participating site.
- Participation in any other interventional study (participation in another observational study is permissible).
- Parents/legal guardians are unable to give consent due to learning or other difficulties.

Recruitment and Informed consent.

The study team will monitor admissions of any women in threatened or established preterm labour. Any identified women will be approached by the study team to discuss and consider the study and verbal consent for participation will be taken. All parents/legal guardians of eligible participants will be approached if the

baby is born and remains eligible for informed written consent. If the person(s) providing consent on behalf of the infant does not speak English, every effort will be made to use translational service to provide an opportunity to participate in the study. If the investigator is not able to confidently take informed consent the infant will not be recruited to the study.

Study Intervention

The recombinant fragment of surfactant protein D drug product has been manufactured to good manufacturing practice (GMP) and is known as rfhSP-D in this study. The IMP has orphan designation with the Food and Drug Administration (FDA). The sterile IMP is formulated in 0.9% saline at a concentration of 1 mg/mL in 2mL vials. The first administration of rfhSP-D will be given as soon as possible after administration of standard-of-care surfactant therapy, this will be known as T0. Subsequent administration will be given at T0+12 (±2) hours and T0+24 (±2) hours. If the infant requires further standard surfactant therapy which coincides with the time of IMP administration, then the IMP should be given after the standard surfactant therapy has been administered. The IMP will be administered via a surfactant giving set that is inserted into the endotracheal tube. If the participant is extubated before any IMP dose is scheduled, then the IMP will not be administered. Vital signs will be monitored every 15 minutes for the first hour after administration of the IMP. The study drug can be administered by any authorised medically trained delegate. Eligibility criteria will be confirmed prior to each administration.

Criteria for discontinuing participation in the trial.

Any dose modifications in this protocol will be in line with the trial design and according to the dose level confirmed by the DSMB and the TSC.

Reasons that the intervention may be discontinued are:

- Any change in the infant's condition that in the clinician's opinion justifies the discontinuation of treatment.
- Withdrawal of consent for treatment by the parent/guardian/legal representative.

Participants who discontinue protocol treatment, for any of the above reasons, will remain in the trial for follow-up and data analysis. The study team do not anticipate problems with intervention adherence given

that RESPONSE is an inpatient-based study. All participants in the study will receive standard neonatal care and there will be no alteration in their clinical management. The study does not require any additional follow-up of the participants recruited once they are discharged from the hospital or reach 40 weeks postmenstrual age. The hospital where the participant is being cared for is responsible for any medical care. The sponsor holds indemnity for any trial-related harm caused to the participant.

Study Outcomes

The primary outcome of this study is to assess the safety profile of rfhSP-D across three dose levels and to identify the RP2D. DLEs will be identified using clinical criteria and grading as described below.

Dose Limiting events (DLE)

The severity of all adverse events (AE) and/or adverse reactions (AR)s (serious and non-serious) in this trial will be graded using the toxicity graded in the NAESS v1.0 (35). The NAESS has been developed as existing scores such as the Common Terminology Criteria for Adverse events (CTCAE) is not suitable for use in a study involving neonates. The NAESS developed by the International Neonatal Consortium has been developed to facilitate the conduct and appropriate interpretation of neonatal clinical trials such as RESPONSE (36).

Grades for neonatal-specific adverse events according to the NAESS v1.0 (35) are:

Grade 1: Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; no change in baseline age-appropriate behaviours*; no change in baseline care or monitoring indicated.

Grade 2: Moderate; resulting in minor changes of baseline age-appropriate behaviour*; requiring minor changes on baseline care or monitoring*+.

Grade 3: Severe; resulting in major changes of baseline age-appropriate behaviour* or non-life-threatening changes in basal physiological processes+ requiring major change in baseline care or monitoring**

Grade 4: Life-threatening; resulting in life-threatening changes in basal physiological processes+; requiring urgent major change in baseline care***.

Grade 5: Death

*Age-appropriate behaviour refers to oral feeding, voluntary movements and activity, crying pattern, social interactions and perception of pain .
+ Basal physiological processes refers to oxygenation, ventilation, tissue perfusion, metabolic stability and organ functioning
** Minor care changes constitute: brief, local, non-invasive or symptomatic treatments

*** Major care change constitute: surgery, addition of long-term treatment, and upscaling care level.

The DSMB will determine the occurrence of a DLE based on the following criteria:

- A single event defined as Grade 3 or above on the NAESS that is possibly, probably or definitely thought to be related to the IMP. Relatedness will be confirmed by an independent neonatologist at the participating site.
- A single serious adverse event (SAE) that is possibly, probably, or definitely thought to be related to the IMP. Relatedness will be confirmed by an independent neonatologist at the participating site.
- **Concerns over frequency** of any adverse events reported at grade 2 on the NAESS that are possibly, probably or definitely thought to be related to the IMP.

Secondary outcomes related to efficacy for this study are:

- Evaluation of systemic absorption of rfhSP-D using serial measurements of SP-D in plasma and its continued presence in tracheal fluid.
- To determine the effect of rfhSP-D on inflammatory markers in the lung secretions (e.g. cell counts of neutrophils, macrophages, matrix metalloproteinases, IL-8,IL-6, IL-1).
- To compare the clinical effects of endotracheal administration of rfhSP-D on physiological and intensive care parameters in treated infants in this trial with non-treated infants from a parallel observational study.

Participant timeline

The first administration of rfhSP-D will be given as soon as possible after administration of standard-of-care surfactant therapy, this will be known as T0. Subsequent administration will be given at T0+12(±2) hours and T0+24(±2) hours. Eligibility and screening investigations will be done before each administration of the IMP as shown in the schedule of events table 1.

Study Visit	Screening	Baseline	Pre	Pre	Pre

			Instillation of IMP T0	Instillation of IMP T0 +12h(±2hrs)	Instillation of IMP T0 +24h(±2hrs)
Informed consent	*				
Eligibility	*				
Clinical stability		*	*	*	*
Demographics (incl. Gestational Age)	*	*			
Pregnancy and delivery history		*			
Stabilisation history		*			
Clinical assessment (anomalies)	*				
Vital signs	*	*		*	*
Oxygen concentration	*	*		*	*
Ventilator modality		*		*	*
Ventilator settings		*		*	*
Haematology (as per Standard of Care)		*			*
Biochemistry (as per Standard of Care)		*			*
Cytokine levels (plasma)		*			*
Cell counts GA/ETA		*		*	*
Surfactant replacement	*				
Plasma SP-D and rfhSP-D levels		*		*	*
Blood gases	*	*		*	*
SP-D levels GA		*			
rfhSP-D and SP-D level ETA				*	*
Concomitant medication		*		*	*
Cranial ultrasound scan		*			*
Review of AEs and SAEs (from time of consent)		*	*	*	*

Table 1. Schedule of events at screening and prior to administration of the IMP. Standard of care refers to blood samples that are taken as part of clinical care and not specific to the study .

Further clinical data will be collected as per the time points outlined in table 2 schedule of events

Study Visit	**T0 +36h (±6h)**	**T0 +48h (±4h)**	**T0 +72h (±12h)**	**T0 +96h (±12h)**	**T0 +7d (±2d)**	**T0+14d (±2d) T0+21d (±2d) T0+28d (±2d)**	**36w PMA (±1d)**	**40w PMA or Hospital Discharge (±1w)**
Vital signs	*	*	*	*	*	*	*	*
Oxygen concentration	*		*	*	*	*	*	*
Ventilator modality	*	*	*	*	*	*	*	*
Ventilator settings	*	*	*	*	*	*	*	*
Haematology (SoC)		*	*	*	*			
Biochemistry (SoC)		*	*	*	*			
Plasma cytokine levels		*	*	*	*		*	
Plasma SP-D and rfhSP-D		*	*	*	*		*	
Blood gases		*	*	*	*	*	*	
ET cell counts	*	*	*	*	*			
ET rfhSP-D and SP-D levels		*	*	*	*		*	
Concomitant medication	*	*	*	*	*	*	*	*
Cranial ultrasound scan		*			*	*	*	*
Walsh Oxygen Test							*	
Review of AEs and SAEs	*	*	*	*	*	*	*	*

Table 2. Subsequent time points in participant timeline following IMP administration. SoC: as per standard of care;

ET: endotracheal aspirate (if remains intubated)

The RESPONSE study will collect clinical data and biological specimens (blood, tracheal and gastric aspirates) as per table 1 and 2. Parents/guardians/those legally responsible for the participant will have the option to give consent for any anonymised data and samples that are collected as part of RESPONSE to be used in other ancillary studies that have ethical approval. Gastric secretions will be taken from all infants at the time of admission after placement of oro/naso-gastric tube and will be discarded if consent is not obtained. Blood samples (0.5mls of blood in an ethylenediaminetetraacetic acid, EDTA microtainer) will be collected at birth, 12 hours, 24 hours, 48 hours, 72 hours, 96 hours, day 7 and at 36 weeks postmenstrual age. Given the risk of anaemia and associated co-morbidities in the study population, if there are any clinical concerns about anaemia, the clinical stability of the infant or the participant is receiving a blood transfusion the blood samples for SP-D and cytokine analysis will either be delayed or not taken.

Data collection and management

Participants once recruited to the RESPONSE study will be allocated a study number so that all data and samples that are taken are anonymized e.g RES_001. Participation in the clinical study will be recorded in the participant clinical records. Participants will be enrolled by the study team on the OpenClinica database. Participant clinical and laboratory data will be entered directly into the password protected study database. After completion of the trial the data will be exported and retained in restricted folders by the sponsor. All data will be held for 10 years following the completion of the trial.

Primary outcome data collection in this study (safety profile of rfhSP-D) will be done through grading and analysis of the incidence of DLEs. Potential causality of the DLE to the IMP will be assessed by an independent neonatologist. In addition to the dose-limiting events the following datasets will be collected from electronic patient records:

- At screening and on eligibility assessment: sex of participant, ethnicity, maternal medical history, antenatal steroid courses, date and time of rupture of membranes, concerns about maternal sepsis, ventilatory requirement on admission and administration of standard exogenous surfactant.

Secondary outcome data collection:

- Data will also be collected at the time points specified in the schedule of events (Tables 2 and 3) and this will include: concomitant medication, ventilatory support and parameters, known positive microbiology, use of postnatal steroids, presence and treatment of pulmonary hypertension, pneumothorax, patent ductus arteriosus, use of postnatal steroids.
- At 36 weeks PMA all participants will be assessed for severity of BPD and data will be collected about complications of prematurity such as episodes of necrotizing enterocolitis and retinopathy of prematurity. The severity of BPD in participants will be defined as per the 2019 NICHD criteria (36), an oxygen reduction test will be done if the participant is eligible (requiring less than Fi02 < 0.3 or 1.1L/min and not on positive pressure support) and remains an inpatient at the recruiting site.

All data will be handled in accordance with the Data Protection Act 2018 and GDPR and all study members will have current GCP training and certification.

Analysis of biological samples

Biological samples will be collected as per the schedule of events. Samples will be labelled with the participant's study number and transported to the Targeted Lung Immunotherapy Laboratory, UCL. Surfactant components, inflammatory markers and level of SP-D will be analysed using the ELISA technique (ELLA, BioTechne) with single marker studies and multiplex assays. Cytokines to be analysed include IL-1β, IL-6, IL-8, IL-11, IL-10, IL-13, matrix metalloproteinase-9 and tumour-necrosis factor-α. Cell counts (lymphocytes, neutrophils and macrophages) in gastric and tracheal aspirates will be assessed using flow cytometry. Samples will be retained if consent has been given by the parent/legal guardian for 5 years for use in any other ethics-approved studies. If consent is not given for use in further studies or at the end of 5 years, the biological samples will be destroyed as per the laboratory standard operating procedure.

Sample size and Statistical analysis

As this is a safety study, no formal sample size calculation has been performed. A sample size of 24 infants is planned to meet practical recruitment and time targets and to collect sufficient data to quantify the estimated risk of DLE at each dose level. Participants with unclear safety outcomes or who have not started study treatment will be replaced to meet our planned effective sample size of 24 participants.

The primary outcome of interest is the occurrence of DLEs at the dose levels under investigation and the identification of the dose(s) that, for infants of particular risk profiles defined by gestational age, have an estimated risk of DLE closest to the target side effect level of no greater than 20%. The use of Bayesian methodology to estimate risks will allow information to be borrowed across dose levels, making the dose-escalation and RP2D identification procedure more efficient than a standard rule-based approach.

The operating characteristics of the design, for three specific scenarios, are shown in Table 3. The first scenario is one where the initial a-priori DLE probabilities calculated by calibration (halfwidth of the indifference interval set at 0.05) correspond to the true underlying probabilities of DLE. The second scenario is such that the true DLE rate of the second dose level corresponds to the target DLE rate. The third scenario is one where the true probabilities are much lower than the a-priori probabilities.

Starting dose level 1, dose-skipping not allowed, 3 dose levels, maximum 24 participants.
Skeleton (a-priori probabilities of DLE) = 0.05, 0.11, 0.20
Target DLE rate = no greater than 20%

	Dose level		
	1	**2**	**3**
Scenario 1; recommendation [%]	2.5	24.5	73.0
Scenario 2; recommendation [%]	22.5	46.5	31.0
Scenario 3; recommendation [%]	0.0	3.3	96.7

Scenario 1: true probabilities = a-priori probabilities
Scenario 2: true probabilities = 0.10, 0.20, 0.30
Scenario 3: true probabilities = 0.02, 0.05, 0.10

Table 3. Operating characteristics of the Continuous Reassessment Model

Type and grade of DLEs, SAEs and AEs will be tabulated per dose level, and further summarised by risk group defined by gestational age. Mean estimated risk of DLE per dose level and 95% credibility intervals will be calculated using the study model. Secondary objectives will be described per dose level and risk category. Categorical variables will be summarised by frequencies and percentages, and continuous variables by means/medians and standard deviation/interquartile ranges per dose level.

Interim analyses of dose-limiting events

Interim analysis will done to assess if DLEs have occurred after each infant (for the first 3 participants at each dose level) has received the final dose of IMP and 72 hours of monitoring and all NAESS data will be reviewed by the DSMB. The DSMB will evaluate the safety data before further participants can be recruited, this will

only be for the first three infants at each dose level. The purpose of this is to ensure there are no safety concerns. For the remainder of the study interim analysis will be done after cohorts of three participants are recruited at any dose level to assess the occurrence of DLE and review all clinical data. Overall DSMB review of all data to advise the TSC regarding dose escalation will take place after recruitment of 3 infants at any dose level. The trial statistician will calculate and provide updated summaries of the estimated risk of dose-limiting toxicity at each dose level. The DSMB will then advise if dose escalation can occur. There will be no interim analysis for the secondary outcomes.

The study will be terminated if any of the following stopping rules are satisfied:

- There is at least 90% chance that the risk of DLE at dose level 1 is greater than the target of 20%. If the trial is terminated under this rule, no drug dose will be recommended due to safety concerns.
- The number of participants that have been treated without side effects is deemed sufficient.
- There is evidence of increased mortality or morbidity in the participants treated with the IMP.

Study Oversight and Monitoring

The sponsor will provide trial oversight and verify the trial processes and prompting corrective action to the clinical study team as required. An independent TSC will provide oversight of the trial to safeguard the interests of the trial participants. The TSC will also provide advice to the chief investigator (CI), CCTU and the funder on all aspects of the trial through its independent chair. An independent DSMB is assigned with an allocated chair. The DSMB will be responsible for monitoring and accumulating the safety data and making recommendations to the TSC on whether the trial should continue as planned. The DSMB will consider data as per the statistical analysis and make recommendations to the TSC chair for consideration by the TSC.

Patient and Public Involvement:

The TSC has a patient representative and the patient-facing documents have been reviewed and commented on by the patient representatives.

Adverse event reporting

All adverse events grade 3 or above on the NAESS/ SAE will be reported to CCTU within 24 hours until the participant reaches Day 7 following the last administration of the IMP (pre-clinical studies have demonstrated that the IMP is not detectable in plasma sample taken 24 hours after final administration). All related events that are graded 1 or 2 according to the NAESS will be reported within 7 days. After Day 7 any events that are considered related to the IMP will be reported within 24 hours of knowledge to the sponsor. Assessments for, and reporting of all adverse events related/unrelated will continue until 40 weeks PMA or hospital discharge. All aggregated adverse events data will be considered by the DSMB at each meeting to confirm that there are no trends, safety signals or safety concerns. Examples of adverse events that are exempt from reporting are those that are graded 1 or graded 2 according to the NAESS criteria if considered not related to the IMP. These are common observations in pre-term infants and do not require a change in clinical management unless sustained, i.e. grade 3 and above on the NAESS. There is no formal frequency of audit for this study and but will be overseen by the sponsor and if required by the Medicine and Healthcare products Regulatory Agency.

Significance of Study

Despite the medical advances in neonatal medicine, the incidence of BPD has not changed over the years, and one may argue that it has increased because we see an increasing number of extremely preterm infants survive to discharge. The life-long morbidity associated with BPD has significant implications for healthcare systems around the world.

Infants at highest risk of BPD are born at a gestational age when the majority of the alveolar and vascular development in the lungs occurs. Immaturity of the lungs means they have a developmental deficiency of surfactant leading to RDS. Ongoing lung injury secondary to postnatal insults such as infection and mechanical ventilation leads to ongoing interruption to lung development. Efforts have been made over the year to reduce these insults by changes in ventilation strategies, early nutrition, and proactive management of infection in the hope that the lungs will repair and remodelling will lead to recovery of the lung parenchyma. However, a significant number of preterm infants will have multiple insults and despite best efforts will have abnormal repair with little lung recovery leading to BPD. Inflammation remains at the centre

of the pathophysiology of BPD and the most promising target for therapies. Given this, there is a need for novel anti-inflammatory therapies to be explored such as SP-D.

The role of SP-D in lung immune homeostasis is well established but due to its propensity to oligomerise does not lend itself well to a stable drug form. The proposed recombinant fragment of SP-D has been developed into a stable drug form for endotracheal administration and animal studies in a well-established translational model have demonstrated its potential anti-inflammatory effects. This phase I safety study using dose escalation of 1-4mg/kg will aim to identify a recommended Phase 2 dose for a subsequent randomised Phase 2 study in preterm infants born at less than 28 weeks gestation who are at highest risk of developing neonatal chronic lung disease.

Ethics and Dissemination

All results and analysis from the study will be published in peer-reviewed journals and presented at national and international conferences. All data generated in this study will be anonymised and the study will be conducted per Good Clinical practice. Access to the full study protocol will be given upon request and participant-level data will only be given if authorised by the sponsor for auditing purposes. Any substantial or non-substantial amendment to the study must be approved by the Health Research Authority and will be communicated with the NHS trust research and development team to ensure site implementation. Any study material that is related to participant information or informed consent will be submitted to the principal research ethics committee for approval. This study has been approved by London-Brent NHS Research Health Authority ethics committee (REC reference 23/LO/0381) and has clinical trials approval (CTA 20363/0453/001-0001) in place.

Figure Legends:

Figure 1. Dose escalation process in RESPONSE using rfhSP-D in preterm infants at risk of BPD.

Figure 2. Schematic overview of the dose escalation design using the Continual Reassessment Method. This schematic overview illustrates the decisional pathway planned. The salmon triangle refers to data review by the Data and Safety Monitoring Board (DSMB). which takes place 1) for the first three babies 72h after study entry after each baby at each dose level (and confirms no DLE to the management group

- termed DSMB assessment), 2) after any potential DLE has been observed (and recommends trial action to Trial Steering Committee (TSC)), and 3) in groups of three babies to make a recommendation to TSC about dose escalation. In the case of 2 or 3 a full data review by the DSMNB is undertaken (identified with a 'R"). In the event of a suspected DLE, the DSMB will be consulted to recommend to the TSC to continue at the same dose/reduce dose and continue or trial needs to be terminated. If a DLE occurs, then the DSMB may recommend recruitment at the same dose level (e.g. 'grey" participants) or they may advise dose de-escalation i.e. 2mg/kg to 1mg/kg for a further 3 participants before further review, after which if no DLE occurs they may advise escalation in dose again. In the absence of DLEs the trial will continue to recruit to the same dose level during DSMB /TSC review periods.

Abbreviations

AE: Adverse Events

AR: Adverse Reaction

BAL: Bronchoalveolar Lavage

BPD: Bronchopulmonary dysplasia

CCTU: Comprehensive Clinical Trials Unit

CRM: Continual reassessment method

CTCAE: Common Terminology Criteria for Adverse Events

DLE: Dose Limiting Event

DSMB: Data and Safety Monitoring Board

GA: Gestational Age

IL: Interleukin

IMP: Investigational Medicinal Product

MTD: Maximum Tolerated Dose

NAESS: Neonatal Adverse Event Severity Score

PMA: Post Menstrual Age

rfhSP-D: Recombinant Fragment of Human Surfactant Protein D

RP2D: Recommended phase 2 dose

SAE: Serious Adverse Event

SP: Surfactant Protein

TSC: Trial Steering Committee

UCL: University College London

Acknowledgements

We are grateful to our funders, the Medical Research Council for their ongoing support with this study. We would like to thank the UCL CCTU and the UCL/UCLH Joint Research Office team for their continued support and contribution in RESPONSE trial.

Contributors statement

RB, JM, TC, KC, HMD, NM and HC contributed to the study design and ethics application. RB, JM, and TC planned and wrote the laboratory analysis plan. HMD devised the statistical analysis plan. RB, KC, NM and HC designed and planned the clinical data collection, and designed the clinical case report forms. RB, HC, JM and NM wrote and edited the clinical trial protocol. RB wrote the manuscript. RB, JM, TC,KC, HMD, NM and HC have reviewed and approved the manuscript. HC is the guarantor.

Competing interests

The authors declare they have no competing interests.

Funding Statement:

This work is supported by the Medical Research Council (MRC) DPFS grant, grant number MR/P026907/1.

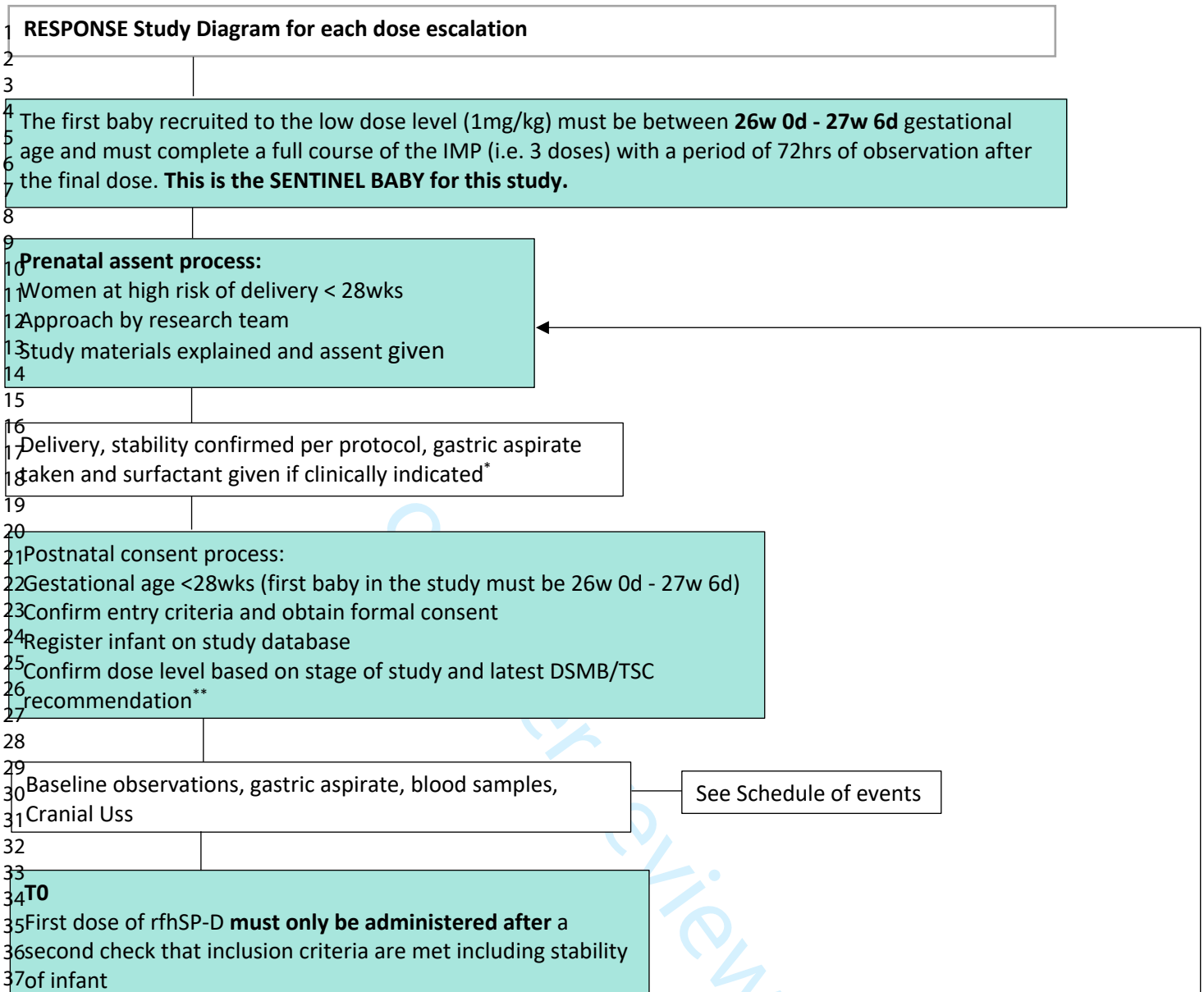
Data Sharing Statement

All trial data will be anonymised and entered on OpenClinica. On completion of the trial data will be available on reasonable request from the sponsor.

References

1. Abman SH, Bancalari E, Jobe A. The Evolution of Bronchopulmonary Dysplasia after 50 Years. American Journal of Respiratory and Critical Care Medicine. 2017;195(4):421-4.
2. JOBE AH, BANCALARI E. Bronchopulmonary Dysplasia. American Journal of Respiratory and Critical Care Medicine. 2001;163(7):1723-9.
3. Siffel C, Kistler KD, Lewis JFM, Sarda SP. Global incidence of bronchopulmonary dysplasia among extremely preterm infants: a systematic literature review. The Journal of Maternal-Fetal & Neonatal Medicine. 2021;34(11):1721-31.
4. Thébaud B, Goss KN, Laughon M, Whitsett JA, Abman SH, Steinhorn RH, et al. Bronchopulmonary dysplasia. Nature Reviews Disease Primers. 2019;5(1):78.
5. Bancalari E, Claure N, Sosenko IRS. Bronchopulmonary dysplasia: changes in pathogenesis, epidemiology and definition. Seminars in Neonatology. 2003;8(1):63-71.
6. Ehrenkranz RA, Walsh MC, Vohr BR, Jobe AH, Wright LL, Fanaroff AA, et al. Validation of the National Institutes of Health consensus definition of bronchopulmonary dysplasia. Pediatrics. 2005;116(6):1353-60.
7. Davidson LM, Berkelhamer SK. Bronchopulmonary Dysplasia: Chronic Lung Disease of Infancy and Long-Term Pulmonary Outcomes. Journal of Clinical Medicine. 2017;6(1):4.
8. Zysman-Colman E, Tremblay G, Bandeali S, Landry S. Bronchopulmonary dysplasia - Trends over three decades. Paediatrics & child health. 2013;18:86-90.
9. Stoll BJ, Hansen NI, Bell EF, Walsh MC, Carlo WA, Shankaran S, et al. Trends in Care Practices, Morbidity, and Mortality of Extremely Preterm Neonates, 1993-2012. Jama. 2015;314(10):1039-51.
10. Costeloe KL, Hennessy EM, Haider S, Stacey F, Marlow N, Draper ES. Short term outcomes after extreme preterm birth in England: comparison of two birth cohorts in 1995 and 2006 (the EPICure studies). BMJ : British Medical Journal. 2012;345:e7976.
11. Hurst JR, Beckmann J, Ni Y, Bolton CE, McEniery CM, Cockcroft JR, et al. Respiratory and Cardiovascular Outcomes in Survivors of Extremely Preterm Birth at 19 Years. American Journal of Respiratory and Critical Care Medicine. 2020;202(3):422-32.
12. Um-Bergström P, Hallberg J, Pourbazargan M, Berggren-Broström E, Ferrara G, Eriksson MJ, et al. Pulmonary outcomes in adults with a history of Bronchopulmonary Dysplasia differ from patients with asthma. Respiratory Research. 2019;20(1):102.
13. Marlow N, Johnson S, Hurst JR. The extremely preterm young adult - State of the art. Semin Fetal Neonatal Med. 2022;27(3):101365.
14. Taghizadeh A, Reynolds E. Pathogenesis of bronchopulmonary dysplasia following hyaline membrane disease. The American journal of pathology. 1976;82(2):241.
15. Jobe AJ. The New BPD: An Arrest of Lung Development. Pediatric Research. 1999;46(6):641-.
16. Schultz C, Temming P, Bucsky P, Göpel W, Strunk T, Härtel C. Immature anti-inflammatory response in neonates. Clinical and experimental immunology. 2004;135(1):130-6.
17. Sullivan G, Galdi P, Borbye-Lorenzen N, Stoye DQ, Lamb GJ, Evans MJ, et al. Preterm Birth Is Associated With Immune Dysregulation Which Persists in Infants Exposed to Histologic Chorioamnionitis. Frontiers in Immunology. 2021;12.
18. Speer CP. Inflammation and bronchopulmonary dysplasia: A continuing story. Seminars in Fetal and Neonatal Medicine. 2006;11(5):354-62.
19. Possmayer F, Yu SH, Weber JM, Harding PG. Pulmonary surfactant. Can J Biochem Cell Biol.

1984;62(11):1121-33.
20. Watson A, Madsen J, Clark HW. SP-A and SP-D: Dual Functioning Immune Molecules With Antiviral and Immunomodulatory Properties. Frontiers in Immunology. 2021;11.
21. Wright JR. Immunoregulatory functions of surfactant proteins. Nature Reviews Immunology. 2005;5(1):58-68.
22. Beresford MW, Shaw NJ. Bronchoalveolar lavage surfactant protein a, B, and d concentrations in preterm infants ventilated for respiratory distress syndrome receiving natural and synthetic surfactants. Pediatr Res. 2003;53(4):663-70.
23. Kotecha S, Davies PL, Clark HW, McGreal EP. Increased prevalence of low oligomeric state surfactant protein D with restricted lectin activity in bronchoalveolar lavage fluid from preterm infants. Thorax. 2013;68(5):460-7.
24. Mackay R-MA, Townsend JP, Calvert J, Anthony M, Wilkinson AR, Postle AD, et al. Increased surfactant protein-D levels in the airways of preterm neonates with sepsis indicated responses to infectious challenges. Acta Paediatrica. 2019;108(5):870-6.
25. Clark H, Reid K. The potential of recombinant surfactant protein D therapy to reduce inflammation in neonatal chronic lung disease, cystic fibrosis, and emphysema. Archives of Disease in Childhood. 2003;88(11):981-4.
26. Clark H, Palaniyar N, Strong P, Edmondson J, Hawgood S, Reid KB. Surfactant protein D reduces alveolar macrophage apoptosis in vivo. J Immunol. 2002;169(6):2892-9.
27. Sato A, Whitsett JA, Scheule RK, Ikegami M. Surfactant Protein-D Inhibits Lung Inflammation Caused by Ventilation in Premature Newborn Lambs. American Journal of Respiratory and Critical Care Medicine. 2010;181(10):1098-105.
28. Knudsen L, Ochs M, Mackay R, Townsend P, Deb R, Mühlfeld C, et al. Truncated recombinant human SP-D attenuates emphysema and type II cell changes in SP-D deficient mice. Respir Res. 2007;8(1):70.
29. Sorensen GL, Hoegh SV, Leth-Larsen R, Thomsen TH, Floridon C, Smith K, et al. Multimeric and trimeric subunit SP-D are interconvertible structures with distinct ligand interaction. Mol Immunol. 2009;46(15):3060-9.
30. Strong P, Reid KBM, Clark H. Intranasal delivery of a truncated recombinant human SP-D is effective at down-regulating allergic hypersensitivity in mice sensitized to allergens of Aspergillus fumigatus. Clinical and experimental immunology. 2002;130(1):19-24.
31. Various A. Selected Abstracts of the 12th International Congress of UENPS; Krakow (Poland); September 2nd-4th, 2022. Journal of Pediatric and Neonatal Individualized Medicine (JPNIM). 2022;12(1):e120104.
32. O'Quigley J, Pepe M, Fisher L. Continual reassessment method: a practical design for phase 1 clinical trials in cancer. Biometrics. 1990;46(1):33-48.
33. Faries D. Practical modifications of the continual reassessment method for phase i cancer clinical trials. Journal of Biopharmaceutical Statistics. 1994;4(2):147-64.
34. Goodman SN, Zahurak ML, Piantadosi S. Some practical improvements in the continual reassessment method for phase I studies. Stat Med. 1995;14(11):1149-61.
35. Salaets T, Turner MA, Short M, Ward RM, Hokuto I, Ariagno RL, et al. Development of a neonatal adverse event severity scale through a Delphi consensus approach. Arch Dis Child. 2019;104(12):1167-73.
36. Jensen EA, Dysart K, Gantz MG, McDonald S, Bamat NA, Keszler M, et al. The Diagnosis of Bronchopulmonary Dysplasia in Very Preterm Infants. An Evidence-based Approach. American Journal of Respiratory and Critical Care Medicine. 2019;200(6):751-9.



*via Endotracheal tube only
**First iteration will be at 1mg/kg/dose, subsequent iterations 2mg/kg/dose and 4mg/Kg/dose
***Neonatal Adverse Event Severity Score (NAESS)
****administration window of +2hrs

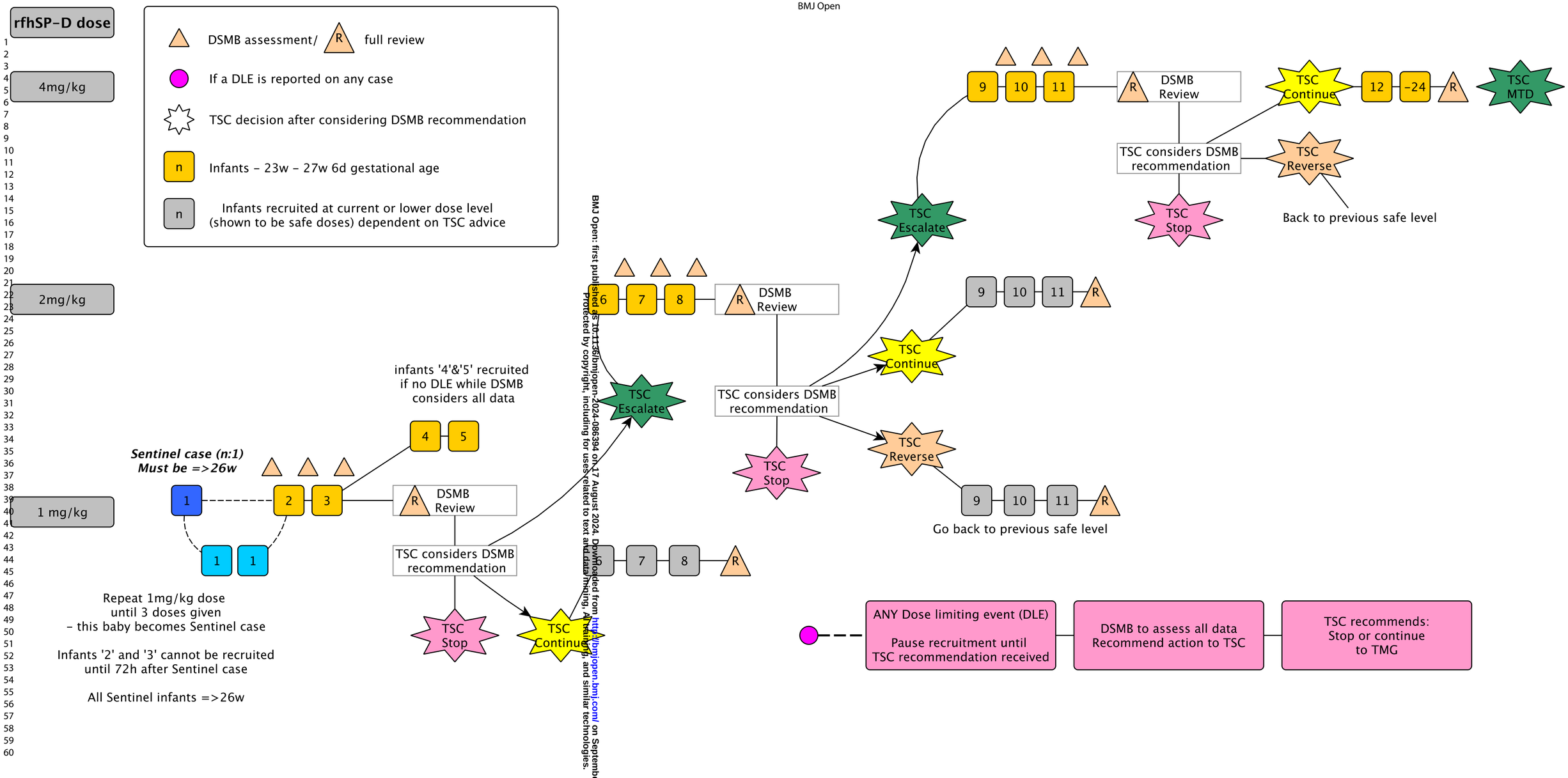


Figure 12: Schematic example of the dose escalation design using the Continual Reassessment Method. The salmon triangle refers to data review by the DSMB. which takes place
1) for the first three babies 72h after study entry after each baby at each dose level (and confirms no DLE to the management group - termed DSMB assessment),
2) after any potential DLE has been observed (and recommends trial action to TSC), and
3) in groups of three babies to make recommendation to TSC about dose escalation. In the case of 2 or 3 a full data review by the DSMB is undertaken (identified with a 'R").
In the event of a suspected DLE, the DSMB will be consulted to recommend to the TSC to continue at the same dose/reduce dose and continue or trial needs to be terminated.
If a DLE occurs, then the DSMB may recommend recruitment at the same dose level (e.g. 'grey" participants) or they may advise dose de-escalation i.e. 2mg/kg to 1mg/kg for a further 3 participants before further review, after which if no DLE occurs they may advise escalation in dose again. In the absence of DLEs the trial will continue to recruit to the same dose level during DSMB /TSC review periods.

Reporting checklist for protocol of a clinical trial.

Based on the SPIRIT guidelines.

Instructions to authors

Complete this checklist by entering the page numbers from your manuscript where readers will find each of the items listed below.

Your article may not currently address all the items on the checklist. Please modify your text to include the missing information. If you are certain that an item does not apply, please write "n/a" and provide a short explanation.

Upload your completed checklist as an extra file when you submit to a journal.

In your methods section, say that you used the SPIRITreporting guidelines, and cite them as:

Chan A-W, Tetzlaff JM, Gøtzsche PC, Altman DG, Mann H, Berlin J, Dickersin K, Hróbjartsson A, Schulz KF, Parulekar WR, Krleža-Jerić K, Laupacis A, Moher D. SPIRIT 2013 Explanation and Elaboration: Guidance for protocols of clinical trials. BMJ. 2013;346:e7586

		Reporting Item	Page Number
Administrative information			
Title	#1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	#2a	Trial identifier and registry name. If not yet registered, name of intended registry	3
Trial registration: data set	#2b	All items from the World Health Organization Trial Registration Data Set	3
Protocol version	#3	Date and version identifier	3
Funding	#4	Sources and types of financial, material, and other support	3
Roles and responsibilities: contributorship	#5a	Names, affiliations, and roles of protocol contributors	1 and 28

Roles and responsibilities: sponsor contact information	#5b	Name and contact information for the trial sponsor	3
Roles and responsibilities: sponsor and funder	#5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	3
Roles and responsibilities: committees	#5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	22
Introduction			
Background and rationale	#6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	4
Background and rationale: choice of comparators	#6b	Explanation for choice of comparators	N/A this is a phase I study and there is no control or comparator.
Objectives	#7	Specific objectives or hypotheses	8
Trial design	#8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, non-inferiority, exploratory)	8
Methods: Participants, interventions, and outcomes			

Study setting	#9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	8
Eligibility criteria	#10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	12
Interventions: description	#11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	13
Interventions: modifications	#11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving / worsening disease)	14
Interventions: adherance	#11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return; laboratory tests)	14
Interventions: concomitant care	#11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	13/14
Outcomes	#12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	14
Participant timeline	#13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	16
Sample size	#14	Estimated number of participants needed to achieve study objectives and how it was	20

		determined, including clinical and statistical assumptions supporting any sample size calculations	
Recruitment	#15	Strategies for achieving adequate participant enrolment to reach target sample size	13
Methods: Assignment of interventions (for controlled trials)			
Allocation: sequence generation	#16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	n/a - this is an open-labelled safety trial. All participants will be anonymised with a study number that will be sequential as they are recruited to the study.
Allocation concealment mechanism	#16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	n/a – this is not a blinded study but a safety phase I study.
Allocation: implementation	#16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	18
Blinding (masking)	#17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	n/a - this is a Phase I safety study with only one intervention group and there will be no blinding of the intervention.
Blinding (masking): emergency unblinding	#17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	n/a - this is a Phase I safety study.

Methods: Data collection, management, and analysis			
Data collection plan	#18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	18
Data collection plan: retention	#18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	20
Data management	#19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	18
Statistics: outcomes	#20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	20
Statistics: additional analyses	#20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	n/a - there is only one group in this protocol and therefore no subgroup analyses will be done.
Statistics: analysis population and missing data	#20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	20

Methods: Monitoring			
Data monitoring: formal committee	#21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	22
Data monitoring: interim analysis	#21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	21
Harms	#22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	22
Auditing	#23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	22
Ethics and dissemination			
Research ethics approval	#24	Plans for seeking research ethics committee / institutional review board (REC / IRB) approval	23
Protocol amendments	#25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC / IRBs, trial participants, trial registries, journals, regulators)	23
Consent or assent	#26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	13

Consent or assent: ancillary studies	#26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	16
Confidentiality	#27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	19
Declaration of interests	#28	Financial and other competing interests for principal investigators for the overall trial and each study site	25
Data access	#29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	19
Ancillary and post trial care	#30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	15
Dissemination policy: trial results	#31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	23
Dissemination policy: authorship	#31b	Authorship eligibility guidelines and any intended use of professional writers	23
Dissemination policy: reproducible research	#31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	23
Appendices			
Informed consent materials	#32	Model consent form and other related documentation given to participants and authorised surrogates	Appendix 1
Biological specimens	#33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or	20

molecular analysis in the current trial and for future use in ancillary studies, if applicable