The efficacy and mechanism of surfactant therapy for critically ill infants with bronchiolitis:
The **B**ronchiolitis **E**ndotracheal **S**urfactant **S**tudy (BESS)

EudraCT No. 2018-001169-18

Trial registration: ISRCTN 11746266

Statistical Analysis Plan – Final V2.0 16/04/2024

	UPDATED & APPROVED BY
Name	Ashley Jones
Title	Head of Statistics
Date	16/04/2024
Protocol Version and Date	V6.0 09/10/2023

1. Change Control

Protocol version	Updated SAP version no.	Section number changed	Description of change	Date changed
6.0	2.0	20	The TSC recommended the addition of a sub-group analysis following their meeting on 20th February 2024.	16/04/2024

2. Approval and agreement

SAP Version Number being approved: 2.0

Trial Statistician

Name N/A

Signed _____ Date _____

Senior Statistician

Name Dr Ashley Jones

Signed AP Jones box SIGN 42YWJYX1-1VW5Z73Z Date 16 Apr 2024

Chief Investigator/clinical lead

Name Professor Malcom (Calum) Gracie Semple OBE

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OR Electronic approval attached ☐

3. Roles and responsibilities

Name	Affiliation	Role
Ashley Best (AB)	Liverpool Clinical Trials Centre (LCTC), University of Liverpool	Trial Statistician
Dr Ashley Jones (AJ)	Liverpool Clinical Trials Centre (LCTC), University of Liverpool	Senior Statistician
Professor Malcom (Calum) Gracie Semple OBE (MGS)	Women's and Children's Health, University of Liverpool	Chief Investigator

Version 1.0

AJ and AB proposed the statistical analysis plan. AB drafted the statistical analysis plan. AJ and MGS read, amended and approved the statistical analysis plan.

Version 2.0

The TSC recommended a sub-group analysis be added to the statistical analysis plan. The TSC made this decision without any knowledge of treatment allocation.

AJ drafted the statistical analysis plan. AJ and MGS read, amended and approved the statistical analysis plan.

List of abbreviations and definitions of terms

AE	Adverse event
CRF	Case report form
ES	Effective Shunt
ET	Endotracheal tube
GCP	Good Clinical Practice
LRSQ	Liverpool Respiratory Symptom Questionnaire
MV	Mechanical ventilation
NIV	Non-invasive ventilation
OI	Oxygenation Index
OSI	Oxygenation Saturation Index
PICU	Paediatric Intensive Care Unit
SBT	Spontaneous breathing test
SF	SpO_2/FiO_2 ratio
VI	Ventilation Index

4. Contents

5. Statement of Compliance

This Statistical Analysis Plan (SAP) provides a detailed and comprehensive description of the pre-planned final analyses for the study “BESS”. The planned statistical analyses described within this document are compliant with those specified in brief within the BESS protocol v3.0 (31/07/2019). These analyses will be performed by the trial statistician.

This study is carried out in accordance with the World Medical Association Declaration of Helsinki (1964) and the Tokyo (1975), Venice (1983), Hong Kong (1989) and South Africa (1996) amendments and will be conducted in compliance with the protocol, Liverpool Clinical Trials Centre (LCTC) Clinical Trials Unit (CTU) Standard Operating Procedures (SOPs) and EU Directive 2001/20/EC, transposed into UK law as the UK Statutory Instrument 2004 No 1031: Medicines for Human Use (Clinical Trials) Regulations 2004.

This study is a clinical trial of a medicinal product and is registered on the EudraCT database. This SAP has been developed to support the posting of results on the EudraCT system. This is a regulatory requirement which should be fulfilled within 6 months after the end of the study as defined within the clinical trial protocol. The results of the final analysis described within this SAP will be contained within a statistical analysis report. This report will be used as the basis of the primary research publications according to the study publication plan.

All analyses are performed with standard statistical software (SAS v9.4 or later) with the exception of the joint modelling analysis which will be performed using the JoineR package [5] package in R. The finalised analysis datasets, programs and outputs will be archived following Good Clinical Practice (GCP) guidelines and SOP GE012 Archiving procedures. The testing and validation of the statistical analysis programs will be performed following SOP ST001.

6. Background and Rationale

Detailed background and rationale can be found in Section 3 of the BESS protocol. In brief, bronchiolitis is the single most common reason for hospital admission of infants (children < 1-year-old). There is no vaccine or specific treatment. Breathing fails in very severe cases and intensive care is required, with breathing supported by a mechanical ventilator. Studies of infants with life-threatening bronchiolitis show reduced lung compliance and surfactant deficiency. The aim of the trial is to investigate whether giving endotracheal surfactant reduces the time babies with bronchiolitis spend on a mechanical ventilator.

7. BESS Study Objectives

The overall purpose of the trial is to investigate whether endotracheal surfactant reduces the duration of mechanical ventilation (MV) when given to infants early in the course of critical illness due to

bronchiolitis. BESS is a superiority trial with a primary outcome of duration of MV. Hypotheses are as follows:

- Null hypothesis: There is no difference in time on MV between treatment arms.
- Alternative hypothesis: The intervention arm (endotracheal surfactant) will reduce the duration of MV compared to placebo.

Secondary objectives are detailed in the BESS protocol Section 3.6.

8. Investigational Plan and Study Design

8.1. Overall study design and plan- description

BESS is a multi-centre, blinded, randomised, placebo-controlled phase II trial with a parallel group design.

8.2. Treatments studied

- Endotracheal poractant alfa (Curosurf®, porcine lung phospholipid fraction supplied at 80mg/mL), first dose 200mg/kg, repeated 12 hourly at 100mg/kg, up to a total of three doses according to the manufacturer's summary of product characteristics.
- An identical series of up to three procedures (while intubated) using air as the placebo.

8.3. Treatment compliance

The number of doses and volume of treatment drawn up and administered will be noted in the hospital chart and accountability forms, and reasons for treatment not being administered recorded on the trial case report form (CRF).

8.4. Patient population studied

The patient population will include infants aged <26 weeks (or preterm infants with <26 weeks corrected age) with bronchiolitis requiring MV via tracheal intubation.

8.4.1. Inclusion criteria

The inclusion criteria can be found in Section 6.1 of the BESS protocol.

8.4.2. Exclusion criteria

The exclusion criteria can be found in Section 6.2 of the BESS protocol.

8.4.3. Removal of patients from therapy or assessment

Participants may be withdrawn from trial intervention for any of the following reasons:

- Parent withdraws consent
- Unacceptable toxicity
- Intercurrent illness preventing further treatment
- Any change in the participant’s condition that justifies the discontinuation of treatment in the clinician’s opinion.

Follow-up and data collection will continue unless the parent explicitly withdraws consent. Research samples will be destroyed or retained for the purpose of the trial according to the wishes of the parent.

8.5. Consent process

Full details of the consent process can be found in Section 7.2 of the BESS protocol. Consent will be prospective and enrolment must be completed within 48 hours of intubation for MV.

8.6. Blinding

A sham procedure will be used to blind as many research and usual care staff as possible to treatment allocation. The participants, LCTC staff where appropriate, and members of site research teams (with the exception of the randomising staff member, the administrator of the intervention and the dosage witness) will be blinded to allocations.

8.7. Method of assignment to treatment

Participants will be randomised to receive either poractant alfa or air placebo in a 1:1 ratio. The randomisation list will be generated by an independent statistician at the LCTC who is not otherwise involved in the BESS trial. Randomisation lists will be computer-generated using block randomisation, stratified by site and duration of ventilation (<24hrs and >=24hrs) prior to randomisation.

8.8. Sequence and duration of all study periods

Clinical follow-up (daily data collection) will take place for the period of time between randomisation and discharge from hospital. All adverse events will be collected until 24 hours after the participant receives their final trial intervention. Non-serious adverse events occurring after 24 hours post-final intervention but before 90 days post-randomisation/discharge home/death will be reported if the local investigator believes that they may be related to the intervention.

Parent-reported outcomes will be collected via a brief questionnaire collecting hospital readmissions at 3 months post randomisation and the Liverpool Respiratory Symptom Questionnaire (LRSQ) at 6 and 12 months post-randomisation for participants recruited in seasons 1 and 2, and at 6 months only for participants recruited in season 3. The end of follow-up definition for each participant will therefore be 12 months post-randomisation for participants in season 1 and 2, or 6 months post-randomisation for participants in season 3.

8.9. Schedule of assessments

See BESS protocol Section 8.3.

9. Listing of Outcomes

9.1. Primary outcome(s)

Total duration of MV (hours) from randomisation to final extubation, including any time off MV due to failed extubation.

9.2. Secondary outcomes

- Time from randomisation to meeting criteria for readiness for spontaneous breathing test (SBT)
- Number of trial interventions given
- Change from baseline of ventilation index, oxygenation index, and oxygenation saturation index whilst on MV
- Change from baseline of SpO_2/FiO_2 (SF) ratio
- Duration of oxygen supplementation
- Use of steroids to assist extubation
- Duration of post-extubation non-invasive respiratory support
- Duration of stay at Paediatric Intensive Care Unit (PICU) and in hospital
- Failure to administer intervention due to any adverse event (AE) during preparatory processes
- Failure to complete administration of intervention due to any AE during administration of intervention
- Incidence of pneumothorax or pneumomediastinum before discharge from PICU
- Any need to replace the endotracheal tube
- Parent-reported readmission to hospital
- Death during PICU admission
- All-cause mortality at 90 days post-randomisation
- Liverpool Respiratory Symptom Questionnaire (LRSQ) score

9.3. Exploratory outcomes

- Comparison of effective shunt with change from baseline of ventilation index, oxygenation index, and oxygenation saturation index whilst on MV. This outcome is not covered by this analysis plan.
- predictive validity of effective shunt: [9].

10. Determination of Sample Size

The sample size calculation is described in full in Section 11.3 of the BESS protocol.

11. Study Framework

The overall objective of the BESS trial is to test the superiority of endotracheal surfactant compared to an air-placebo sham procedure in all outcomes.

12. Confidence Intervals, p-values and Multiplicity

Confidence intervals will be reported for all point estimates at the 95% level. Baseline summaries will be presented to 1 decimal place whilst all other values will be reported to 2 decimal places, or 1

significant figure if it would round to zero at 2 decimal places, and all p-values will be reported to 3 decimal places. There are no planned adjustments for multiplicity.

13. Timing and Objectives of Interim and Final Analyses

13.1. Interim monitoring and analyses

Interim reports summarising recruitment, withdrawals, baseline characteristics and completeness of data collection will be presented to the trial oversight committees at least annually. There are no planned formal statistical interim analyses.

13.2. Final analysis

The final analysis will be commenced after all participants have completed follow-up and the data are cleaned. The database will be locked once analysis code has been completed and any final queries resolved. The analysis code will then be run on the locked database and the statistical analysis report written.

14. Disposition of Participants

14.1. Screening, eligibility and recruitment

Screening logs will be summarised by site and overall in a table detailing:

i) the number of patients assessed for eligibility
ii) those eligible and parent/person with parental responsibility approached about trial (denominator for percentages being i)
iii) those eligible and parent/person with parental responsibility not approached (denominator for percentages being i)
iv) those eligible and consent obtained (denominator for percentages being ii)
v) those eligible and consent not obtained (denominator for percentages being ii)
vi) those eligible, consented and randomised (denominator for percentages being iv)
vii) those eligible, consented, but not randomised (denominator for percentages being iv)

Reasons for ineligibility, lack of approach and consent being declined will be summarised by site and overall in a table with categories for reasons as defined on the BESS Screening Log CRF.

The final recruitment graph will be presented, showing the predicted and actual number of sites and patients recruited over time. A recruitment summary table will be presented, showing the following for each site: site code, hospital name, dates site opened and closed to recruitment, dates of first and last randomisations and the total number of randomised participants.

A CONSORT flow diagram [4] will be used to summarise the number of patients who were:

- assessed for eligibility at screening
  - eligible at screening
  - ineligible at screening*
- eligible and randomised

- eligible but not randomised*
- received the randomised allocation
- did not receive the randomised allocation*
- discontinued the intervention*
- lost to follow-up*
- randomised and included in the primary analysis
- randomised and excluded from the primary analysis*

*reasons will be provided.

14.2. Post randomisation discontinuations

Reasons for non-administration of trial treatment will be captured on the treatment CRF. Premature discontinuations (treatment no longer being administered when participant still eligible to receive treatment) will be presented and reasons provided. In the event of discontinuation of treatment, participants will continue to be followed-up, unless parents specifically withdraw consent to do so.

15. Protocol Deviations and Serious Breaches of GCP

Protocol deviations are defined in the monitoring plan v1.0 (12/12/2018) and specified as minor or major. The protocol deviation and data set definitions template ST001TEM04 will be completed and approved prior to the release of randomisation codes to the statistical team.

The number of participants experiencing each protocol deviation will be presented, along with the numbers with at least one minor deviation, at least one major deviation and at least one deviation of either classification. No formal statistical testing will be conducted. Any serious of breaches of GCP will be reported with the corresponding corrective and preventative actions.

16. Unblinding

Any unblinding of research staff recorded on the unblinding CRF will be reported. The number of patients for whom treatment allocation was unblinded will be reported for each treatment group; the timepoint, whether the unblinding was accidental and reason(s) as to why they were unblinded will also be reported.

17. Efficacy Evaluations

17.1. Data Sets Analysed

The principle of intention-to-treat, as far as practically possible, will be the main strategy of the analysis adopted for the primary outcome and all secondary outcomes. These analyses will be conducted on all randomised participants, in the group to which they were randomised, and for whom the outcome(s) of interest have been observed/measured. No imputations will be made.

17.2. Demographic and Other Baseline Characteristics

Descriptive statistics will be presented split by treatment group and overall. Categorical data will be summarised by frequencies and percentages to one decimal place. Continuous data will be summarised to one decimal place by mean (standard deviation), median (interquartile range) and

range, irrespective of distribution. No formal statistical testing will be undertaken, but the clinical significance of any imbalance will be noted.

Baseline variables to be included:

- Whether infant premature or term-born
- Age/corrected age at randomisation
- Sex
- Weight
- Whether infant intubated upon arrival at PICU
- Whether infant on oxygen supplementation upon arrival at PICU
- Time on MV prior to randomisation
- Underlying viral/bacterial infection

17.3. Compliance with treatment

Accountability logs and treatment CRFs will be assessed to determine treatment compliance. For each dose, the number of participants expected to receive the dose and the number and percentage actually receiving the full dose will be summarised by allocated treatment group and overall. Reasons for participants not receiving doses will be presented.

17.4. Analysis of efficacy outcomes

17.4.1. Primary Outcome: Total duration of mechanical ventilation

The primary outcome will be estimated by calculating the time from randomisation to final extubation (including any time off MV due to failed extubation) to assess if there is any evidence of a difference in the time between arms.

An attempt to extubate is deemed to have failed if the participant is re-intubated within 48 hours of the most recent extubation. If a participant is re-intubated within 48 hours of the most recent extubation their follow-up should continue until a successful intubation.

17.4.1.1. Derivation

The duration of mechanical extubation will be calculated by identifying the date and time (OAODATXD) associated with final extubation as indicated by OAOOUTCC = “Successful” on the Observations and Outcomes CRF. The difference between the date/time of randomisation taken from the Almac randomisation log and the date/time of successful extubation will be calculated in hours and transformed using natural logarithms.

17.4.1.2. Analysis

Descriptive statistics will be presented split by treatment and overall for the time from randomisation to final successful extubation (before the data is transformed) and time off MV due to failed extubation. The summaries will be presented as median and interquartile range as the data is likely to be skewed.

A comparison of group means will be conducted using a two-sample t-test on the log transformed data (the log transformation will be done using natural logarithms). The between group difference of the log transformed data will then be back transformed by taking the exponent to give the geometric mean difference and the corresponding 95% confidence interval.

A linear mixed effects model will also be fitted on the log transformed data, using ANCOVA to test for a difference between groups with adjustment for the stratification factor of time on ventilation prior to randomisation (<24h and ≥24h), sex and adjusted age at enrolment as a fixed effects and site as a random effect (random intercept only). The exponentiated between-group difference and corresponding 95% confidence interval will be reported.

The approach to the primary outcome analysis will be a compete case analysis and will only include those participants for which the primary endpoint has been observed. If there are any participants who have not recorded a successful extubation at the point at which they were last followed up (e.g. consent for follow up has been withdrawn) then sensitivity analysis will be conducted including these participants to assess the robustness of the primary outcome analysis.

17.4.2. Secondary Outcome 1: Readiness for SBT

This outcome will assess if there is any evidence of a difference in the length of time from randomisation to participant meeting the criteria for readiness for SBT between arms.

17.4.2.1. Derivation

The time to readiness for SBT will be calculated by identifying the date and time (OAODATCD) associated with the observation for which readiness for SBT is first reported as indicated by OAOPSBTC = “Yes” **and** the senior clinician is in agreement with the readiness for SBT as in indicated by the variable OAOCLSTC = “Yes” on the Observations and Outcomes CRF. That is, if the SBT criteria is met numerically (as described in section 8.1.4.1 of the protocol) but the senior clinician is not of the opinion that the infant is ready for an SBT then the infant does not meet the definition of readiness for SBT.

The difference (in hours) between the date/time of randomisation taken from the Almac randomisation log and the date/time of the observation at which readiness for SBT is first reported will be calculated and transformed using natural logarithms.

17.4.2.2. Analysis

Descriptive statistics will be presented split by treatment and overall for the time from randomisation to readiness for SBT (before being transformed). The summaries will be presented as median and interquartile range as the data is likely to be skewed.

The frequency and percentage of the number of times each participant is recorded as meeting the criteria for readiness for SBT will presented split by treatment and overall.

The comparative analysis will be as described in 17.4.1.2 but will just involve the two-sample t-test, a linear mixed effects model will not be used for this outcome.

A sensitivity analysis will be conducted using the first time a patient meets the numerical criteria for SBT. The sensitivity analysis will be as described in 17.4.1.2 but will just involve the two-sample t-test, a linear mixed effects model will not be used for this outcome.

17.4.3. Secondary Outcome 2: Number of trial interventions

This outcome will assess if there is evidence of a difference in the number of trial interventions (both poractant and placebo) given to the infants between trial arms.

17.4.3.1. Derivation

A successful delivery of the intervention is indicated on the Treatment CRF where the MACRO variable TRTINSTC = “Intervention completed”. An infant can receive up to three doses of intervention.

17.4.3.2. Analysis

A two-way table will be presented with the frequency of interventions received vs allocated treatment. A Chi-squared test for trend will be used to test for an association between allocation and the number of trial interventions if the expected cell frequencies are greater than five and Fisher’s exact test will be used if they are not. The Chi-squared test statistic and corresponding p-value will be presented for the test.

17.4.4. Secondary Outcome 3: Change over time from baseline measures

This outcome will assess if there is evidence of a difference between arms in the change over time from baseline to the end of MV for the following measures:

- Ventilation Index (VI)
- Oxygenation Index (OI)
- Oxygenation Saturation Index (OSI)
- SpO_2/FiO_2 (SF) ratio

VI, OI and OSI will be measured by taking capillary blood gas sampling immediately prior to intervention and then at 6, 12, 24, 36 and 48hrs while still on MV and will continue every 48hrs if still on MV.

SF ratio will be reported at the above times while on MV but will also continue post MV and will be recorded every time peripheral O_2 measurement is recorded in routine notes and at least twice in a 24-hour period.

17.4.4.1. Derivation

The required blood gas readings to calculate VI, OI, OSI and SF can be found on the Observations and Outcomes CRF. The following table lists all the required variables.

Blood gas reading	**Description**	**MACRO variable name**
MAP **	Mean airway pressure	OAOMAPI
PIP **	Peak Inspiratory Pressure	OAOPIPI
PEEP **	Positive End Expiratory Pressure	OAOPEEPI
RR	Respiratory rate	OAORSPRI
$PaCO_2$*	Partial pressure of arterial carbon dioxide	OPOPACMC
FiO_2	Fraction of air inspired that is oxygen	OAOFIO2I
PaO_2*	partial pressure of arterial of oxygen	OAOPAO2I
SpO_2	peripheral oxygen saturation	OAOSPO2I
pH	Hydrogen ion concentration (acidity or alkalinity)	OAOPHRR

The following formulas will be required to calculate the blood gas indices:

- VI = ($PaCO_2$ × (PIP-PEEP) × RR)/1,000
- OI = (MAP × FiO_2 × 100)/PaO_2
- OSI = (MAP × FiO_2 × 100)/SpO_2
- SF = SpO_2/FiO_2

****Note:*** $PaCO_2$ and PaO_2 can be recorded in units of either mmHg or kPa. To conduct the analysis all measurements in kPa will be converted to mmHg using the following formula [6]

1 mmHg = 0.133 kPa

Example conversion: *30 kPa = 30 / 0.133 = 225.6 mmHg*

****Note:*** MAP, PIP and PEEP can be recorded in units of either cmH_2O or mbar. To conduct the analysis all measurements in cmH_2O will be converted to mbar using the following formula [6]:

1 cmH_2O = 0.981 mbar

Example conversion: *5 cmH_2O = 5 x 0.981 = 4.903 mbar*

Effective Shunt is an estimate of the proportion of cardiac output that would have to bypass the lungs entirely to obtain these blood gas results at a steady state. The predictive validity of ES in critically ill adults has been reported but this has not yet been explored as a useful indicator in the paediatric population.

17.4.4.2. Analysis

Summary statistics will be presented in a table for each time point (0 hours, 12, hours, 24 hours, 36 hours, 48 hours then every 24 hours while still on MV if the number of observations is ≥ 5 overall at the time points following 48 hours) detailing the mean and standard deviation of each of the derived measures (VI, OI and OSI) for each treatment arm and overall.

The relationship between longitudinal data for VI, OI and OSI and the event of successful extubation will be examined visually by plotting mean profile plots of longitudinal data separated by treatment group and by the time at which the final observation was observed and comparing this to the overall mean profile plot separated by treatment group.

Infants will have differing amounts of follow-up data for the outcomes VI, OI and OSI as these are recorded while on MV only. More specifically, infants who are successfully extubated earlier (and therefore completing follow-up earlier) will have less follow-up data than infants with a later successful extubation time. This will be addressed in analyses of both the longitudinal measurements (VI, OI and OSI) and the time to event outcome (time to successful extubation) using joint modelling. Joint modelling will account for the potentially informative drop-out in longitudinal measures due to successful extubation. For each measure the treatment effect will be reported together with a 95% CI and a p-value.

For the SF ratio, a repeated measures random effects model will be fitted. The dependent variable will be post baseline (0 hours) SF ratio measurements. Covariates will be: SF ratio at baseline (0 hours), treatment arm, time (fitted as a continuous variable), and the stratification factor of time on ventilation prior to randomisation (<24h and ≥24h). Centre will be fitted as a random intercept. The treatment effect (estimated mean difference in SF ratio) will be reported together with a 95% CI and a p-value.

17.4.5. Secondary Outcome 4: Duration of oxygen supplementation

This outcome will assess if there is any evidence of a difference between groups in the duration of post-extubation oxygen supplementation of any kind, this includes all forms of non-invasive ventilation (NIV) and/ or high or low-flow oxygen.

17.4.5.1. Derivation

The post-extubation oxygen supplementation details can be found on the Post Mechanical Ventilation follow up CRF. The start point for the duration of post-extubation oxygen supplementation will the date and time of final successful extubation as described in section 17.4.1.1, if this has been observed.

The end point will be the final date and time (MACRO variable PMV02DTD) that any type of oxygen supplementation (NIV[1], high-flow O_2[2] or low-flow O_2[3]) is ended as indicated by the final observation for which the MACRO variable PMVS02SC (Supplementary O2 stopped?) = “Yes”.

If oxygen supplementation is stopped and then restarted this time will be included in the overall time of post-extubation respiratory support. If no oxygen supplementation is recorded then time on post-extubation respiratory support will be assumed to be zero.

17.4.5.2. Analysis

Descriptive statistics will be presented split by treatment and overall for the time from successful extubation to cessation of post-extubation oxygen supplementation. The summaries will be presented as median and interquartile range as the data is likely to be skewed.

The comparative analysis will be as described in 17.4.1.2 but will just involve the two-sample t-test, a linear mixed effects model will not be used for this outcome.

17.4.6. Secondary Outcome 5: Use of steroids to assist extubation

This outcome will assess if there is a difference in the use of steroids to assist extubation between arms.

17.4.6.1. Derivation

The details for steroid use during extubation can be found on the Observations and Outcomes CRF indicated where the variable OAOSTERC= “Yes”.

17.4.6.2. Analysis

This binary outcome will be presented in a two-way frequency table (allocated treatment group by use of steroids during extubation) and a Chi-squared test will be used to test association between allocation and use of steroids if the expected cell counts a greater than five and Fisher’s exact test will be used if they are not. The Chi-squared test statistic and corresponding p-value will be presented for the test.

The relative risk between arms of having to use steroids during extubation will be presented along with 95% confidence interval.

17.4.7. Secondary Outcome 6: Duration of post-extubation respiratory support

This outcome will assess if there is any evidence of a difference between groups in the duration of post-extubation non-invasive respiratory support, this includes all forms of non-invasive ventilation (BiPAP +/- ASB, CPAP +/- ASB, CPAP +/- pressure support).

[1] BiPAP +/- ASB, CPAP +/- ASB, CPAP +/- pressure support

[2] AIRVO, Optiflow, Vapotherm

[3] Nasal cannula

17.4.7.1. Derivation

The post-extubation NIV respiratory support details can be found on the Post Mechanical Ventilation follow up CRF. The start point for the duration of post-extubation respiratory support will the date and time of final successful extubation as described in section 17.4.1.1, if this has been observed.

The end point will be the final date and time (MACRO variable PMV02DTD) that NIV is ended as indicated by the final observation for which the MACRO variable PMVTOSPC (Type of Supplementary O2) = “NIV”.

If NIV is stopped and then restarted this time will be included in the overall time of post-extubation respiratory support. If no NIV is recorded then time on post-extubation respiratory support will be assumed to be zero.

17.4.7.2. Analysis

Descriptive statistics will be presented split by treatment and overall for the time from successful extubation to cessation of post-extubation respiratory support. The summaries will be presented as median and interquartile range as the data is likely to be skewed.

The comparative analysis will be as described in 17.4.1.2 but will just involve the two-sample t-test, a linear mixed effects model will not be used for this outcome.

17.4.8. Secondary Outcome 7: Duration of stay at PICU and in hospital

The outcome will assess if there is evidence of a difference in the duration of stay in PICU and in hospital between arms.

17.4.8.1. Derivation

The duration of stay at PICU will be calculated by taking the difference between the date/ time of randomisation taken from the Almac randomisation log and the date/ time of discharge from PICU (PDAISDTD) on the PICU Discharge CRF or the date of death [4](DNBDIDD) Day 90 Status CRF if the death occurred before PICU discharge. This can be expressed as:

$$T(PICU) = \begin{cases} \text{PDAISDTD}, & if\ dicharged \\ \text{DNBDIDD}, & if\ death\ occurs\ in\ PICU \end{cases} - T_{RAND}$$

The overall duration of stay in hospital (including both time in PICU and time in hospital) will be calculated by taking the difference between the date/ time of randomisation taken from the Almac randomisation log on and the date/ time of hospital discharge (PDA90DTD) on the PICU or the date of

[4] If date and time of death is required to calculate time in hospital or time in PICU then this will be estimated at 12:00 as death is only recorded as date.

death (DNBDIDD) Day 90 Status CRF if death occurred before hospital discharge. This can be expressed as:

$$T(Hospital) = \begin{cases} \text{PDA90DTD}, & if\ dicharged \\ \text{DNBDIDD}, & if\ death\ occurs \end{cases} - T_{RAND}$$

Note that if death occurs during PICU then $T(Hospital) = 0$.

17.4.8.2. Analysis

Descriptive statistics will be presented split by treatment and overall for duration of PICU stay and hospital stay separately (before they have been transformed). The summaries will be presented as median and interquartile range as the data is likely to be skewed.

The comparative analysis will be as described in 17.4.1.2 for the duration of PICU stay and hospital stay, but will just involve the two-sample t-test, a linear mixed effects model will not be used for this outcome.

17.4.9. Secondary Outcome 8: Liverpool Respiratory Symptom Questionnaire

The "Liverpool Respiratory Symptom Questionnaire" (LRSQ) score, a parent reported outcome measure of respiratory symptoms, at 6m (+/-1m) and 12m (+/-1m) [6]. The parents of infants randomised in season 3 will only complete the 6m questionnaire.

17.4.9.1. Derivation

The LRSQ will be captured in the Form 2 6 Months LRSQ and Form 3 12 Months LRSQ in JotForm. The LRSQ consists of eight domains, each containing between three and five items. The first six domains assess respiratory symptoms and the remaining two assess the impact of symptoms on the child and family. Parents will be asked to consider symptoms over the last 3 months, with questions being scored on a five-point Likert scale from "not at all" (score 0) to "every day" (score 4). A score for each domain and for the complete questionnaire will be calculated (maximum score 128) [6].

The domains and the symptoms they assess are listed in the table below:

Questionnaire domain	**Symptoms assessed**
1. Daytime symptoms 2. Symptoms with colds 3. Interval symptoms (between colds) 4. Symptoms with activity	Cough, wheeze, shortness of breath, "rattly" chest
5. Night-time symptoms	Cough, wheeze, shortness of breath, "rattly" chest, snoring
6. Other symptoms	Noisy breathing not from chest, noisy breathing from throat, fast breathing
7. Effects on child	Feeding, activity levels, sleep disturbance, fatigue

8. Effects on family	Family activities, adjustment to family life, disturbed sleep, worry/anxiety

17.4.9.2. Analysis

Descriptive statistics will be presented split by treatment and overall for LRSQ scores for 6 months, 12 months and the difference (12-month – 6-month) where available. The summaries presented will be the number, number missing, mean and standard deviation.

A linear mixed effects model will be fitted using ANCOVA to test for a difference between groups 12-month LRSQ score with adjustment for 6-month LRSQ score and the stratification factors of time on ventilation prior to randomisation (<24h and ≥24h) as a fixed effects and site as a random effect (random intercept only). The between-group difference and corresponding 95% confidence interval will be reported.

This will be a complete case analysis and only fully completed questionnaires will be included in the analysis.

17.5. Analysis of safety outcomes

The analysis of the following safety outcomes should follow the ITT principle.

17.5.1. Safety Outcome 1: Failure to administer intervention due to AE

This outcome will assess if there is a difference in the occurrences of the failure to administer the intervention due to an AE. This is can occur in two possible ways:

- Failure to administer the intervention due to any adverse event during preparatory processes (tracheal toilet or BAL)
- Failure to complete administration of the intervention due to any adverse event during administration of the intervention (regardless of which arm of allocation)

Adverse events recorded from randomisation up to final trial intervention will be the period when this outcome could be observed.

17.5.1.1. Derivation

The details of failure to administer intervention can be found on the Treatment CRF.

A failure to administer the intervention due to any adverse event during preparatory processes (tracheal toilet or BAL) is indicated where the variable TRTINSTC=” Intervention not done” and the variable TRTISTRC = “F - Adverse Event during preparatory process”.

A failure to complete administration of the intervention due to any adverse event during administration of the intervention is indicated where the variable TRTINSTC=” Intervention not completed” and TRTISTRC is one of (“E - Adverse Event”, “G - Serious Adverse Event”).

17.5.1.2. Analysis

The total number of occurrences of failure to administer intervention due to adverse event will be presented overall and for both treatment groups and also the number of patients in each group will be reported.

A two-way table of the frequency failed intervention delivery per infants by allocated treatment group will be presented. The comparative analysis between arms will be as described in section 17.4.3.2.

17.5.2. Safety Outcome 2: Incidence of air leak

This outcome will assess if there is a difference between the groups in the number of incidents of 'air leak' (pneumothorax and pneumomediastinum) occurring between randomisation and discharge from PICU.

17.5.2.1. Derivation

The occurrence of any air leaks is recorded on the PICU Discharge/ Day 90 Status CRF and is indicated where the variable PDAIRLKC = "Yes" and the corresponding frequency of these events is expressed by the variable PDALINCI. All occurrences will be summed within patients.

17.5.2.2. Analysis

A two-way table of the frequency of events of air leak per infants by allocated treatment group will be presented. The comparative analysis between arms will be as described in section 17.4.3.2.

17.5.3. Safety Outcome 3: Parent reported readmission to hospital

Parent reported readmission to hospital (all causes) up to 90 days post randomisation

17.5.3.1. Derivation

The outcome is captured on JotForm Form 1 90-day Readmission and the frequency is captured in the column: "How many times was your child admitted to hospital in the 3 months after they took part in BESS?" and can take values from zero to six or more.

17.5.3.2. Analysis

A two-way table of the frequency of parent reported readmission to hospital by allocated treatment group will be presented. The comparative analysis between arms will be as described in section 17.4.3.2.

17.5.4. Safety Outcome 4: Any need to replace the endotracheal tube

This outcome will assess if there is a difference in the frequency of the need to replace the endotracheal tube (ET) between randomisation and successful extubation.

17.5.4.1. Derivation

The details for whether the ET tube needs to be replaced can be found on the Observations and Outcomes CRF indicated where the variable OAOCETTC = "Yes".

17.5.4.2. Analysis

This outcome will be analysed as described in section 17.4.6.2, the two-way table will be allocated treatment group by replacement of ET tube required and the relative risk will be of the tube having to be replaced.

17.5.5. Safety Outcome 5: Death during PICU admission

This outcome will assess if there is a difference in the occurrence of death from randomisation to PICU discharge.

17.5.5.1. Derivation

Death during PICU admission and all-cause mortality at 90 days after randomisation will be recorded on the Day 90 Status CRF indicated when the variable DNBTBAC = “No”. All-cause mortality at Day 90 will be established via a local enquiry against the hospital electronic health record and will be recorded on the Day 90 status CRF.

This variable DNBTBAC (is the infant believed to be still alive?) will be marked as “No” whether the death occurred in the PICU or at any point up to the completion of the 90 days status CRF (including during any hospital admission following PICU admission). To establish if the death occurred during PICU stay, the PICU discharge CRF will be cross checked and if the date/ time of PICU discharge (PDA90DTD) is not completed then the death will be assumed to have occurred during PICU stay and if it is complete then the death will be assumed to have occurred either in hospital or following discharge from hospital up to day 90 post randomisation.

17.5.5.2. Analysis

This outcome will be analysed as described in section 17.4.6.2, the two-way table will be allocated treatment group by whether the infant died during PICU (which will be established as described in section 17.5.5.1) and the relative risk will be of death occurring.

17.5.6. Safety Outcome 6: All-cause mortality

This outcome is to assess if there is a difference in all-cause mortality at 90 days post randomisation between arms. This outcome will be assessed from PICU discharge to 90 days post randomisation.

17.5.6.1. Derivation

See section 17.5.5.1 for details on how to derive this outcome.

17.5.6.2. Analysis

This outcome will be analysed as describe in section 17.4.6.2, the two-way table will be allocated treatment group by whether the infant died during after PICU stay and before 90 days post randomisation (which will be established as described in section 17.5.5.1) and the relative risk will be of death occurring.

18. Missing data and withdrawals

Missing data will be quantified for all outcomes presented in the final analysis report. Where known, reasons for missingness will be given.

Withdrawals from trial will be summarised and presented as line listings detailing:

1. Site
2. Time from randomisation to withdrawal
3. Decision to withdraw participant made by:
    a. Clinician
    b. Parent/person with parental responsibility
    c. Both clinician and parent/person with parental responsibility
4. Reason for withdrawal:
    a. Death
    b. Lost to follow-up
    c. Transfer to non-participating site
    d. Withdrawal of consent for follow-up
    e. Other (specify)

Every effort will be made to collect follow-up data even in the event of participants discontinuing the trial intervention. In the case of withdrawals from study, available data will still be included using an intent-to-treat approach.

19. Additional analyses

Additional analysis 1

A sensitivity analysis of the primary analysis will be done to assess the robustness of the primary analysis. This will be done using a Cox proportional hazards model and will allow the censoring of patients who do not observe the primary endpoint of successful extubation. The start-point and end point for patients having the event of successful extubation will be the same as those defined in section 17.4.1.1. For patients who do not have a successful extubation recorded these will be censored at the last date and time (OAODATXD) an observation is recorded on the Observations and Outcomes CRF. The cox proportional hazards model will include the allocated treatment and time on ventilation prior to randomisation as covariates.

Additional analysis 2

A subgroup analysis of the primary outcome will be undertaken, This was based on a recommendation from the Trial Steering Committee who met on the 20th February 2024.

The subgroup analysis will examine the primary outcome stratified by whether the baby was born prematurely or not and will include an interaction term between treatment and prematurity in the linear mixed effect model described in 18.4.1.2.

Results will be presented from the model with the interaction result alongside.

20. Safety Evaluations

20.1. Data sets analysed

Participants who received at least one dose of either intervention or placebo will be included in the safety analysis dataset. Results will be presented by the treatment that participants actually received, rather than that to which they were allocated.

20.2. Presentation of the data

All AEs reported by the clinical investigator will be presented, with separate tables for serious and non-serious events. Both the number of events and the number of patients experiencing each event will be provided. A third table will be presented grouping non-serious AEs by severity; participants will be counted in the most severe category experienced if they experienced more than one. Similarly, a fourth table will be presented grouping non-serious ARs by causality (where the relationship to intervention is recorded as possibly, probably or almost certainly) and participants will be counted in the highest relationship category experienced if they experienced more than one.

The above tables for serious and non-serious events will be repeated but will be further grouped by whether or not the patient received muscle relaxant prior to the administration of the intervention. Serious adverse reactions will be presented as line listings, detailing the description, seriousness, severity, expectedness, relationship to intervention and outcome.

Relationship to study drug will be assessed first assuming the participant is in the intervention arm, then assuming they are in the control arm, so as to avoid unnecessary unblinding. However, for the classification of causality only the relationship to the intervention received will be reported.

For all AE descriptions, MedDRA classifications will be used. Events will be reported using the preferred term, categorised by system organ class. Results will be presented descriptively; no formal statistical testing will be undertaken for interim reports.

21. Quality Control

To ensure quality control, an independent statistician will follow this SAP to independently program the primary analysis and safety analysis from the raw data. Any discrepancies found will be discussed with the trial statistician to resolve. No programming will be shared or shown between the statisticians. The independent statistician will also check the report against their output obtained from the statistical software.

22. References

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