OPTIMIST-A TRIAL

MULTICENTRE RANDOMISED CONTROLLED TRIAL OF MINIMALLY-INVASIVE SURFACTANT THERAPY IN PRETERM INFANTS 25-28 WEEKS GESTATION ON CONTINUOUS POSITIVE AIRWAY PRESSURE

Statistical Analysis Plan
Version 1, 30-Oct-2020

Document Version History

Version Date	Version	Author	Signature	Change Description	Reason/Comment
30-Oct-2020	Version 1	Francesca Orsini		Initial release.	Not applicable.

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LIST OF ABBREVIATIONS

BPD	Bronchopulmonary Dysplasia
CEBU	Clinical Epidemiology and Biostatistics Unit
CI	Confidence Interval
CPAP	Continuous Positive Airway Pressure
CRF	Case Report Form
FiO_2	Fraction of Inspired Oxygen
HF	High Flow
HR	Heart Rate
IQR	Interquartile Range
ITT	Intention-To-Treat
IVH	Intraventricular Haemorrhage
MCRI	Murdoch Children's Research Institute
MIST	Minimally-Invasive Surfactant Therapy
MV	Mechanical Ventilation
NEC	Necrotising Enterocolitis
NICU	Neonatal Intensive Care Unit
PP	Per Protocol
PVL	Periventricular Leukomalacia
RDS	Respiratory Distress Syndrome
ROP	Retinopathy of Prematurity
RR	Relative Risk
SAE	Serious Adverse Event
SD	Standard Deviation
SpO_2	Haemoglobin Oxygen Saturation measured by Pulse Oximetry
TSC	Trial Steering Committee

1. STUDY OBJECTIVES

1.1. PRIMARY OBJECTIVE

To evaluate in a randomised controlled trial the efficacy of surfactant delivery via a minimally-invasive technique in preterm infants 25-28 weeks gestation with respiratory distress syndrome (RDS) treated with continuous positive airway pressure (CPAP).

2. BACKGROUND/INTRODUCTION

2.1. STUDY DESIGN

Multicentre, blinded, parallel group randomised controlled trial aiming to randomise 606 infants in >25 Neonatal Intensive Care Units (NICUs).

2.2. TREATMENT GROUPS

After assessment of eligibility as per section 2.3, and with parental consent, infants are randomised in a 1:1 allocation ratio to receive minimally-invasive surfactant therapy (MIST) followed by return to support with CPAP, or to remain on CPAP with no surfactant administration (control group). The intervention (MIST or control) is blinded from clinical staff. A treatment team not immediately engaged in clinical management performs the intervention behind screens, taking care not to reveal which group the infant was allocated to. Parents and outcome assessors are also blinded to the group allocation.

2.3. STUDY POPULATION

Preterm infants of gestation 25 weeks 0 days to 28 weeks 6 days who are inborn and admitted to the NICU of a participating study centre, and who fulfil the following eligibility criteria:

Inclusion criteria:

1. Requiring CPAP (or nasal intermittent positive pressure ventilation) because of respiratory distress.
2. CPAP pressure of 5-8 cm H_2O and FiO_2 ≥0.30.
3. Less than 6 hours of age.
4. Agreement of the Treating Physician in charge of the infant's care.

Exclusion criteria:

- Previously intubated, or in imminent need of intubation because of respiratory distress, apnoea or persistent acidosis.
- Congenital anomaly or condition that might adversely affect breathing.
- Identifiable alternative cause for respiratory distress (e.g. congenital pneumonia or pulmonary hypoplasia).
- Lack of availability of an OPTIMIST-A treatment team.

2.4. INTERVENTION

Once randomised, it is expected that the trial intervention will be undertaken within 60 min. Infants randomised to MIST receive surfactant (Curosurf™, Chiesi Farmaceutici, Parma, Italy) administered via the Hobart method[1] at a dosage of 200 mg/kg. Laryngoscopy, tracheal catheterisation and surfactant administration are performed with CPAP prongs remaining *in situ*. The surfactant (volume 2.5 mL/kg bodyweight) is instilled in 2-4 boluses over 15-30 seconds, with the instillation catheter then removed and CPAP reinstituted. If the first attempt at catheterisation fails, the laryngoscope is briefly removed to allow recovery on CPAP before a further attempt. The maximum number of catheterisation attempts is three, after which the procedure is abandoned.

Infants randomised to the control group with no surfactant administration remain on CPAP, receiving no intervention other than a change to body position.

2.5. SAMPLE SIZE

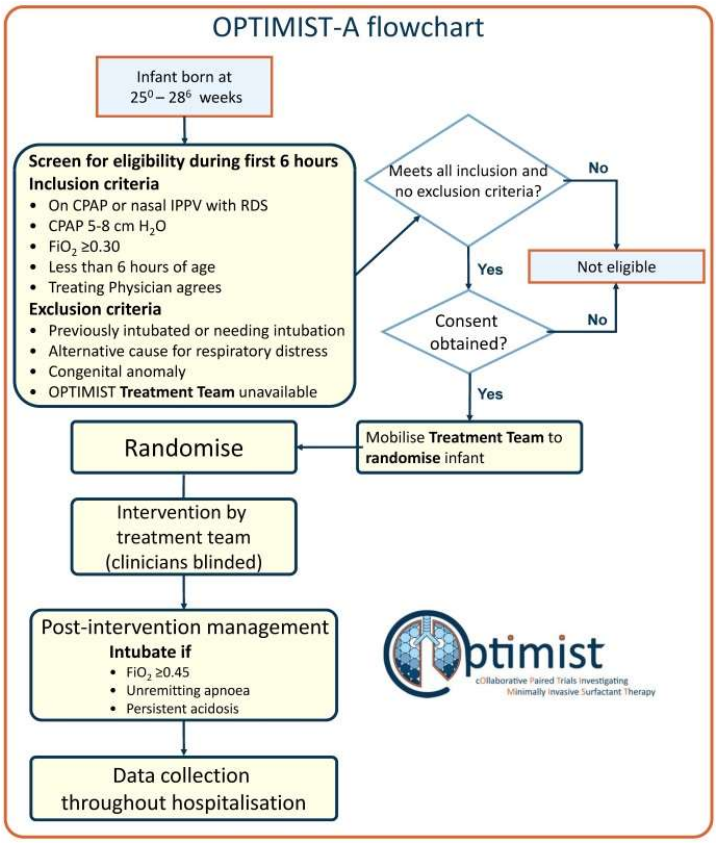
In a previous two-centre observational study,[2] infants of 25-28 weeks gestation supported on CPAP from the outset and reaching OPTIMIST-A enrolment thresholds (CPAP level 5-8 cm H_2O, FiO_2 ≥0.30) in the first 2 h were found to have an incidence of death or bronchopulmonary dysplasia (BPD) of 38%. A reduction by one-third in the proportion of infants with this outcome (i.e. absolute risk reduction 13%, from 38% to 25%) was considered clinically important for this patient group. Detection of a reduction of this magnitude with 90% power and $\alpha = 0.05$ (two-sided) would require 297 subjects per group.[3] An allowance was made for withdrawal of 2% of subjects post-recruitment. The number of subjects to be randomised in each group was thus 303, for an overall total of 606.

The trial protocol allowed for an analysis based on the outcome of requirement for intubation <72 hours, i.e. the same outcome as for the trial of Göpel et al,[4] if large scale funding was not obtained.

The trial commenced in December 2011, with only 25 infants enrolled in 3 vanguard centres during the first 24 months until large-scale funding was secured and the necessary trial infrastructure established. The rate of recruitment reached 100 infants/yr during the calendar years 2016-2018, and slowed to 80/yr in 2019. Research activities were put in abeyance in many participating Units in early 2020 in the wake of the COVID-19 pandemic, leaving only 10 active centres recruiting. The Trial Steering Committee (TSC) found no alternative but to cease enrolment at this juncture, with 486 infants randomised. Recruitment in the trial ceased on 26th March 2020. The TSC noted that, with the observed frequency of death or BPD of 49.8% from the most recent interim analysis (455 infants, *04 Mar 2020*), the recruitment total of 486 gives ~82% power to detect a 13% absolute risk reduction in this primary outcome, $\alpha = 0.05$.[3]

2.6. STUDY PROCEDURE

Figure 1. Flowchart of study procedures



3. POPULATIONS OF ANALYSIS

The **intention-to-treat** (ITT) population will be used in the analyses as the primary efficacy analysis population. Participants will be compared according to the group to which they were randomly allocated, regardless of compliance, crossover to other treatments or withdrawal from the study. This approach preserves the prognostic balance in the study groups achieved by randomisation. The ITT population will EXCLUDE:

- Infants randomised and immediately recognised to be ineligible
- Infants in whom the randomisation failed and the treatment allocation was not revealed to the Treatment Team at the site
- Infants whose parents/guardian withdrew their consent to be in the study and the use of all the data collected

In addition to the intention-to-treat analyses, we will conduct an **as-treated** analysis and a **per protocol** (PP) analysis.

In the **as-treated** analysis data from all the participants will be analysed according to the intervention they received (which may not reflect the group they were originally randomised to), for the primary outcome (death or BPD) and its components, and for the MIST intervention procedural and safety outcomes. This analysis will exclude those infants already excluded from the ITT analysis.

Finally, we will conduct a **per protocol** analysis for the primary outcome (death or BPD) and its components, which will include participants in whom eligibility assessment, randomisation and intervention have been carried out according to the protocol. Infants will be included if they were i) eligible for study entry based on review of Case Report Form (CRF) data, ii) randomised according to the prescribed method, iii) received the intervention they were allocated to in the randomisation, and iv) have primary outcome data available.

4. OUTCOME VARIABLES

4.1. PRIMARY OUTCOME

Table 1 – Primary outcome and its components (*Populations: ITT, As-Treated, PP*)

#	Outcome	Description
1	Primary outcome: Incidence of the composite outcome of **death** before 36 weeks gestation or **physiological BPD*** assessed at 36 weeks corrected gestational age (refer to Figure 1)	Binary outcome 1= Death or physiological BPD at 36 weeks 0= No death and no physiological BPD
Components of the primary outcome:		
1a	Death by 36 weeks corrected gestational age (all causes)	Binary outcome 1= Death by 36 weeks 0= No death by 36 weeks
1b	**Physiological BPD*** in survivors to 36 weeks corrected gestational age	Binary outcome, in those who survived to 36 weeks corrected gestational age (refer to Figure 1) 1= BPD at 36 weeks 0= No BPD by 36 weeks

*Physiological BPD assessed at 36 weeks gestation, defined as:

- Receiving mechanical respiratory support (defined as MV†, CPAP or HF‡ ≥2 L/min); *or (if not on mechanical respiratory support):*
- Receiving crib/head box oxygen with actual FiO_2 ≥0.30 or nasal cannula oxygen with effective FiO_2 ≥0.30; *or*
- Receiving oxygen with actual/effective FiO_2 <0.30 (or nasal cannula air with flow < 2L/min) and failed air trial; *or*
- Receiving oxygen with FiO_2 <0.30 and no air trial performed by physician request

†MV: mechanical ventilation
‡HF: nasal high flow

4.2. SECONDARY OUTCOMES

Table 2: KEY CLINICAL AND SAFETY OUTCOMES (*Population: ITT*)

#	Outcome	Description
2	Air leak requiring drainage	Binary outcome 1=Yes 0=No
3	Need for intubation <72 h	Binary outcome 1=Yes 0=No
4	Severe intraventricular haemorrhage (IVH) – grade III or IV	Binary outcome 1=Yes 0=No
5	Death or major morbidity. Any of: - death during first hospitalisation; - IVH grade III or IV; - cystic periventricular leukomalacia (PVL); - retinopathy of prematurity (ROP) stage 3 or greater; - physiological BPD [as for outcome 1b]	Binary outcome 1=Yes 0=No
6	Death during first hospitalisation (all causes)	Binary outcome 1=Yes 0=No
7	Major morbidity, in survivors. Any of: - IVH grade III or IV; - cystic PVL; - retinopathy of prematurity (ROP) stage 3 or greater; - physiological BPD [as for outcome 1b]	Binary outcome, in those who survived first hospitalisation. 1=Yes 0=No

Table 3: SECONDARY OUTCOMES (*Population: ITT*)

	Outcome	Description
8	Total number of surfactant doses. Includes the dose, if given, during the trial intervention	Continuous outcome (likely to be not normally distributed)
9	Requirement for surfactant via endotracheal tube (ETT)	Binary outcome 1= Yes, if surfactant doses given via ETT is > 0 0 = No
10	Pulmonary haemorrhage	Binary outcome 1= Yes 0 = No
11	Oxygen therapy at day 28	Binary outcome 1= Yes 0 = No
12	BPD (clinical definition), in survivors to 36 weeks gestation	Binary outcome, <u>in survivors to 36 weeks gestation</u> 1 = Yes, BPD present using the clinical definition, diagnosed if oxygen and/or respiratory support (intubation / CPAP / nasal HF ≥2 L/min) is being administered for any portion of the day at 36 weeks and 0 days corrected gestational age. 0 = No, otherwise
13	Mechanical respiratory support at 36 weeks	Binary outcome 1= Yes, if MV, CPAP or HF ≥2 L/min at 36 weeks 0=No, otherwise
14	PDA requiring medical therapy	Binary outcome 1= Yes 0=No
15	IVH any grade	Binary outcome 1= Yes 0 = No
16	Cystic PVL	Binary outcome 1= Yes 0 = No
17	Necrotising enterocolitis (NEC, modified Bell stage 2 or greater)	Binary outcome 1= Yes 0 = No
18	NEC requiring surgery	Binary outcome 1= Yes 0 = No

Table 3: SECONDARY OUTCOMES (*cont.*)

19	Spontaneous intestinal perforation	Binary outcome 1= Yes 0 = No
20	ROP (stage 3 or greater)	Binary outcome 1= Yes 0 = No
21	Late onset sepsis	Binary outcome 1= Yes 0 = No
22	Intubation at any time	Binary outcome 1= Yes 0 = No
23	Days of mechanical ventilation (MV)	Continuous outcome
24	Days of CPAP	Continuous outcome
25	Days of MV+CPAP	Continuous outcome
26	Days of all forms of respiratory support (MV, CPAP, nasal HF)	Continuous outcome
27	Days to regain birth weight	Continuous outcome
28	Days of hospitalisation	Continuous outcome
29	Oxygen therapy at home	Binary outcome 1= Yes 0 = No

Table 4: MIST INTERVENTION PROCEDURAL AND SAFETY OUTCOMES (*Population: As-Treated*)

n.	Outcome	Description
30	Successful administration of surfactant during the procedure	Binary outcome 1= Yes 0= No
31	Premedication used	Categorical outcome 1= Atropine 2= Sucrose 4= Other 0= None
32	Number of catheterisation attempts	Categorical outcome 1/ 2 /3
33	Surfactant dose administered (mg/kg)	Continuous outcome
34	Surfactant reflux observed	Binary outcome 1= Yes 0= No
35	Undue discomfort	Binary outcome 1= Yes 0= No
36	Bradycardia with heart rate <100 beats per minute	Binary outcome 1= Yes, if duration bradycardia > 0 s 0= No, if duration bradycardia = 0 s
37	Bradycardia for more than > 10 seconds	Binary outcome 1= Yes, if duration of bradycardia> 10 s 0= No, if duration bradycardia <= 10 s
38	Hypoxaemia with SpO_2 <80%	Binary outcome 1= Yes, if duration of hypoxaemia > 0 0 = No, if duration of hypoxaemia = 0
39	Hypoxaemia for more than > 30 seconds	Binary outcome 1= Yes, if duration of hypoxaemia > 30 s 0= No, if duration of hypoxaemia <= 30 s
40	Positive pressure inflations required	Binary outcome 1= Yes, if duration of positive pressure inflations > 0 s 0= No, if duration of positive pressure inflations = 0 s
41	Duration of positive pressure inflations (min)	Continuous outcome, in those where duration of positive pressure inflations > 0 s.
42	Procedure duration (min)	Continuous outcome Difference between treatment completion date/time and treatment commencement date/time
43	Emergent intubation required	Binary outcome 1= Yes 0=No

Table 4: MIST INTERVENTION PROCEDURAL AND SAFETY OUTCOMES (*cont.*)

44	Intubation within 1 h	Binary outcome 1= Yes, if intubated within 1 h of MIST procedure 0=No, otherwise
45	Pre-post ΔFiO_2	Continuous outcome FiO_2 at 4 hrs minus pre-intervention FiO_2, determined for infants not intubated at 4 h post-intervention
46	Treatment ineffective	Binary outcome 1= Yes, defined as no improvement in FiO_2 at 4 h post-intervention (infant still supported with CPAP), or intubated <4h post-intervention with FiO_2 above intubation threshold 0 = No (otherwise)
47	Pre-post ΔCO_2 (mm Hg)	Continuous outcome PCO_2 at 4 h post-intervention minus PCO_2 pre-intervention, determined for infants not intubated at 4 h post-intervention
48	Pre-intervention observations: - CPAP level (cm H_2O) - FiO_2 - SpO_2 (%) - Heart rate (beats per min)	Continuous outcome
49	4 h post-intervention observations: - CPAP level in cm H_2O - FiO_2 - SpO_2 (%) - Heart rate (beats per min)	Continuous outcome. In the case of CPAP level and FiO_2, data can only be used if still on CPAP at 4 h.
50	Time of intervention (hours of age and time in minutes after randomisation) (both groups)	Continuous outcome
51	Catheter type	Categorical outcome 1= 16G Angiocath 2= Custom catheter
52	Type of laryngoscope	Categorical outcome 1= Standard 2= Videolaryngoscope

4.3. OTHER PARAMETERS

The following information is collected in relation to the first hospitalisation:

Demographic characteristics

- Gestation (weeks and days)
- Age at randomisation (hrs)
- Birth weight (g)
- Infant sex
- Plurality, birth order (singleton; first of multiples; second or subsequent multiple)

Peripartum details

- Exposure to antenatal glucocorticoids (complete; incomplete; none)
- Delivery mode (vaginal delivery; Caesarean delivery with labour; Caesarean delivery; no labour)
- Apgar score at 5 min

Clinical state at randomisation

- CPAP level at randomisation (cm H_2O)
- FiO_2 at randomisation
    - FiO_2 30-35%
    - FiO_2 >35%

4.4. SERIOUS ADVERSE EVENTS

- Number of events, number of affected infants
- Number with:
    - Unexpected death
    - Life-threatening deterioration
    - Medical occurrence that may prolong hospitalisation
    - Medical occurrence likely to result in persistent and significant disability or incapacity
    - Medical occurrence that could become serious if untreated
- Relationship of SAE to participation in the OPTIMIST-A trial (unrelated; possibly related; probably related; definitely related)
- Occurrence of SAE during the trial intervention

5. STATISTICAL METHODOLOGY

5.1. GENERAL METHODOLOGY

Data analysis for this study will be performed by Ms Francesca Orsini, an experienced biostatistician who works in the Clinical Epidemiology and Biostatistics Unit (CEBU) at the Murdoch Children's Research Institute (MCRI), under Prof. John Carlin's supervision.

The details of the randomisation groups will be unblinded only once the database has been locked and the Statistical Analysis Plan has been finalised, approved by the TSC and appended to the web-based clinical trial registration for the OPTIMIST trial (Australian New Zealand Clinical Trials Registry).

The demographic characteristics, peripartum details and clinical state at randomisation of the infants will be presented for each treatment group using the mean and standard deviation (SD) or median and interquartile range (IQR) for continuous data and using proportions for categorical data.

Multiple outcomes will be considered in evaluating the effectiveness of the trial intervention, along with key composite outcomes (as listed in Table 1, Table 2 and Table 3). The magnitude of potential treatment effect, with 95% confidence interval and p-value, will be estimated for each outcome, but these results will not be interpreted dichotomously against any specific statistical threshold. Instead, findings concerning multiple secondary outcomes will be interpreted cautiously and in context with one another rather than in isolation. Patterns and consistency in the responsiveness of outcomes, and the overall balance of the evidence, will be examined rather than isolated findings that may well be due to chance.

MEASURES OF TREATMENT EFFECT

Relative Risk (RR) between active and control groups will be the measure of treatment effect for binary outcomes.
Mean difference between treatment groups will be the measure of treatment effect for continuous outcomes whose distribution is reasonably symmetrical (see more details in section 5.3).
Median difference between treatment groups will be the measure of treatment effect for continuous outcomes whose distribution is severely skewed (see more details in section 5.3).

ESTIMATION OF TREATMENT EFFECTS

Treatment effects will be estimated using regression methods (generalised linear models, GLM, or quantile regression models) to adjust for the stratification factor and in secondary analyses for additional covariates that are expected to be associated with the primary outcome, for all the outcomes described in Section 4.1 and 4.2. In particular, the following two models will be run.

Primary analysis: adjusted only for gestational age stratum

The treatment effect will be estimated in accordance with the type of estimand under examination (GLMs for relative risk and mean difference, quantile regression for median difference), and adjusted for the gestational age stratum (25-26 weeks; 27-28 weeks) used during randomisation as a covariate. The method will incorporate a cluster-robust standard error calculation to account for clustering by study site.

Secondary analysis: adjusted

For the primary outcome and its components only, the GLM/quantile regression specification above will be extended to include further adjustment for the following covariates, which are expected to be associated with the primary outcome:

- birth weight <10th percentile
- sex
- mode of delivery
- plurality
- antenatal glucocorticoid exposure
- 5-minute Apgar score

HANDLING OF MISSING DATA

It is expected that the proportion of missing data will be very small, therefore the available case analysis will be the primary one.

SENSITIVITY ANALYSES

Sensitivity 1 – As-Treated analysis, population as described in section 3

This sensitivity analysis will be run on the primary outcome and its components, and the MIST intervention procedural and safety outcomes (listed in results Table 4).

Sensitivity 2 –Per-protocol analysis, population as described in section 3

This sensitivity analysis will only be run on the primary outcome and its components.

SUB-GROUP ANALYSES

Sub-Group analysis 1 – Gestational age at birth

Baseline information and all the outcomes (primary and its components, key clinical and safety outcomes, secondary outcomes, MIST intervention procedural and safety outcomes) will be presented in supplementary tables by gestation strata (25-26 weeks; 27-28 weeks). This sub-group analysis will examine the evidence for differences in the effect of the intervention between the gestational age sub-groups. The specific sub-group estimates and confidence intervals will be presented, together with the p-value for interaction, as a guide to how strongly the effects seem to be differentiated. As we have not powered the trial to consider sub-groups, this analysis is considered exploratory.

Sub-Group analysis 2 – FiO_2 at study entry

Sub-groups: (1) FiO_2 0.30-0.35; (2) FiO_2 >0.35

High FiO_2 is a well-recognised proxy for severity of RDS and this analysis will explore whether the intervention has differential effects for infants with higher FiO_2 (>0.35) versus infants with lower FiO_2 (0.30 - 0.35) at randomisation.

This sub-group analysis will be performed for the primary outcome and its components and key clinical and safety outcomes. This sub group analysis will examine the evidence for differences in the effect of the intervention between the sub-groups. The specific sub-group estimates and confidence intervals will be presented, together with the p-value for interaction, as a guide to how strongly the effects seem to be differentiated. As we have not powered the trial to consider sub-groups, this analysis is considered exploratory.

Sub-Group analysis 3 – Geographic region

Acknowledging the worldwide variation in the baseline rates of the primary outcome and its components, and the influence this may have on the observed treatment effect, a third sub-group analysis will be performed by geographical region.

The regions identified are:

1) Australia/NZ/Singapore: 157 participants, 9 sites
2) North America: 102 participants, 9 sites
3) Middle East, 136 participants, 7 sites
4) Europe inc. UK, 90 participants, 8 sites

This analysis will be performed for the primary outcome and its components only. The specific regional estimates and confidence intervals derived by GLMs will be presented graphically, together with the p-value for interaction, as a guide to how strongly the effects seem to be differentiated. In the results, these 4 regions will not be identified by their geographical location, instead by the rate of primary outcome and its components in the control group. This sub-group analysis will examine the evidence for differences in the effect of the intervention between the regions. The specific sub-group estimates and confidence intervals will be presented, together with the p-value for interaction, as a guide to how strongly the effects seem to be differentiated. As we have not powered the trial to consider sub-groups, this analysis is considered exploratory.

5.2. PRIMARY DATA ANALYSES

Primary outcome: composite outcome of **death before 36 weeks gestation** *or* **physiological BPD assessed at 36 weeks corrected gestational age** *(to be reported in results Table 1, outcome 1).*

Results will be summarised as the number and proportion of infants who either died before 36 weeks gestation or have physiological BPD at 36 weeks corrected gestational age in the two treatment groups.

The primary analysis of the primary composite outcome:
[ITT, adjusted only for randomisation strata] The relative risk with 95% confidence internal (CI) will be estimated using a generalised linear model (the "modified Poisson" approach of Zou) to adjust for the gestational age stratum (25-26 weeks; 27-28 weeks) used during randomisation as a covariate. The GLM approach will use the **Poisson** family and employ a **log** link function. The method will incorporate a cluster-robust standard error calculation to account for clustering by study site.

Secondary analyses of the primary composite outcome:
[ITT, adjusted] A secondary analysis of the primary composite outcome will be performed on the ITT population. The RR with 95% CI will be estimated using a GLM (the "modified Poisson" approach of Zou) to adjust for the gestational age stratum (25-26 weeks; 27-28 weeks) as well as a number of **covariates known to have influence on the rate of the primary outcome**, such as birth weight <10th percentile, sex, mode of delivery, plurality, antenatal glucocorticoid exposure, and 5-minute Apgar score. The method will incorporate a cluster-robust standard error calculation to account for clustering by study site.

[Sensitivity 1=As-Treated, adjusted only for randomisation strata] Another secondary analysis of the primary composite outcome will be on the **As-Treated** population. The RR with 95% CI will be estimated using a GLM (the "modified Poisson" approach of Zou) to adjust for the gestational age stratum (25-26 weeks; 27-28 weeks) used during randomisation as a covariate. The GLM approach will use the **Poisson** family and employ a **log** link function. The method will incorporate a cluster-robust standard error calculation to account for clustering by study site.

[Sensitivity 2=PP, adjusted only for randomisation strata] A further secondary analysis of the primary composite outcome will be on the **PP** population. The RR with 95% CI will be estimated using a GLM (the "modified Poisson" approach of Zou) to adjust for the gestational age stratum (25-26 weeks; 27-28 weeks) used during randomisation as a covariate. The GLM approach will use the **Poisson** family and

employ a log link function. The method will incorporate a cluster-robust standard error calculation to account for clustering by study site.

The following sub-group analyses will be run on the primary composite outcome:

- Sub-Group analysis 1, by gestation strata (25-26 weeks; 27-28 weeks): [ITT, adjusted only for gestational age, included as a categorical covariate, ie 25 weeks vs 26 week for sub-group 25-26 weeks ; and 27 vs 28 week for sub-group 27-28 weeks], [ITT, adjusted, with 25 weeks vs 26 week for sub-group 25-26 weeks ; and 27 vs 28 week for sub-group 27-28 weeks]
- Sub-Group analysis 2, by FiO_2 level at study entry (FiO_2 0.30-0.35 and FiO_2 >0.35): [ITT, adjusted only for randomisation strata]
- Sub-Group analysis 3, by geographic region: [ITT, adjusted only for randomisation strata]

Primary outcome components: Death by 36 weeks corrected gestational age (all causes) (outcome 1a) and physiological BPD in survivors to 36 weeks corrected gestational age (outcome 1b).

Analogously to the primary composite outcome, absolute and relative frequencies of infants meeting the definition of outcome 1a and 1b will be calculated and presented by treatment group.
RR with 95% CIs will be estimated using a GLM (the "modified Poisson" approach of Zou), employing the Poisson family, a log link function and a cluster-robust standard error calculation to account for clustering by study site. The following sensitivity analyses will be run:

- [ITT, adjusted only for randomisation strata]
- [ITT, adjusted]
- [Sensitivity 1=As-Treated, adjusted only for randomisation strata]
- [Sensitivity 2=PP, adjusted only for randomisation strata]

The following sub-group analyses will be run on the primary outcome components:

- Sub-Group analysis 1, by gestation strata (25-26 weeks; 27-28 weeks): [ITT, adjusted only for gestational age, included as a categorical covariate, ie 25 weeks vs 26 week for sub-group 25-26 weeks ; and 27 vs 28 week for sub-group 27-28 weeks], [ITT, adjusted, with 25 weeks vs 26 week for sub-group 25-26 weeks ; and 27 vs 28 week for sub-group 27-28 weeks]
- Sub-Group analysis 2, by FiO_2 level at study entry (FiO_2 0.30-0.35 and FiO_2 >0.35): [ITT, adjusted only for randomisation strata]
- Sub-Group analysis 3, by region: [ITT, adjusted only for randomisation strata]

5.3. SECONDARY DATA ANALYSES

Key clinical and safety outcomes (reported in results Table 2, outcomes 2-7)

Since all these outcomes are binary, they will be summarised and analysed similarly to the primary outcome. Particularly, for each outcome, results will be summarised as the number and proportion of infants who meet the outcome in the two treatment groups and comparisons between treatment groups will be made using the following GLMs (the "modified Poisson" approach of Zou, employing the Poisson family, a log link function and a cluster-robust standard error calculation to account for clustering by study site), with derivation of RR and 95% CI, adjusted by the gestational age group used during randomisation, e.g. 25-26 weeks, 27-28 weeks:

- [ITT, adjusted only for randomisation strata]

The following sub-group analyses will be run on all the key clinical and safety outcomes:

- Sub-Group analysis 1, by gestation strata (25-26 weeks; 27-28 weeks): [ITT, adjusted only for gestational age, included as a categorical covariate, ie 25 weeks vs 26 week for sub-group 25-26 weeks ; and 27 vs 28 week for sub-group 27-28 weeks]
- by FiO_2 level at study entry (FiO_2 0.30-0.35 and FiO_2 >0.35): [ITT, adjusted only for randomisation strata]

Binary secondary outcomes (reported in Table 3, outcomes 9-22, 29)

Since all these outcomes are binary, they will be summarised and analysed similarly to the primary outcome. Particularly, for each outcome, results will be summarised as the number and proportion of infants who meet the outcome in the two treatment groups and comparisons between treatment groups will be made using the following GLMs (the "modified Poisson" approach of Zou, employing the Poisson family, a log link function and a cluster-robust standard error calculation to account for clustering by study site), with derivation of RR and 95% CI, adjusted by the gestational age group used during randomisation, e.g. 25-26 weeks, 27-28 weeks:

- [ITT, adjusted only for randomisation strata]

The following sub-group analysis will be run on all the secondary binary outcomes:

- Sub-Group analysis 1, by gestation strata (25-26 weeks; 27-28 weeks): [ITT, adjusted only for gestational age, included as a categorical covariate, ie 25 weeks vs 26 week for sub-group 25-26 weeks ; and 27 vs 28 week for sub-group 27-28 weeks]

Continuous secondary outcomes with not severely skewed distributions (reported in Table 3, outcome 28)

Mean and SD will be calculated and presented by treatment group. The difference in the means between the two treatment groups will be estimated using a GLM approach which will employ a Gaussian family and an identity link and cluster-robust standard error to account for study site. [ITT, Unadjusted Model] will be run and mean differences and 95% CIs will be presented.

The following sub-group analysis will be run on all the secondary continuous outcomes:

- Sub-Group analysis 1, by gestation strata (25-26 weeks; 27-28 weeks): [ITT, adjusted only for gestational age, included as a categorical covariate, ie 25 weeks vs 26 week for sub-group 25-26 weeks ; and 27 vs 28 week for sub-group 27-28 weeks]

Continuous secondary outcomes with severely skewed distributions (reported in Table 3, outcomes 8, 23-27)

Median, interquartile range (IQR) and range will be calculated and presented by treatment group. The differences between the medians with the 95% CI will be estimated using quantile regression models with robust and clustered standard errors, adjusted for the gestational age stratum (25-26 weeks; 27-28 weeks) used during randomisation as a covariate [ITT, adjusted only for randomisation strata].

The following sub-group analysis will be run on all the secondary continuous outcomes:

- Sub-Group analysis 1, by gestation strata (25-26 weeks; 27-28 weeks): [ITT, adjusted only for gestational age, included as a categorical covariate, ie 25 weeks vs 26 week for sub-group 25-26 weeks ; and 27 vs 28 week for sub-group 27-28 weeks]

MIST intervention procedural and safety outcomes (reported in Table 4, outcomes 30-52)

These outcomes will be reported for the MIST group only, on the **As-Treated** population. Outcomes with a not severely skewed distribution will be presented as mean and SD. Continuous outcomes which present a severely skewed distribution will be presented as median, IQR and range. Categorical outcomes will be reported as absolute and relative frequencies.

The following **sub-group analysis** will be run:

- Sub-Group analysis 1, by gestation strata (25-26 weeks; 27-28 weeks): [Sensitivity 1=As-Treated, only descriptive analysis]

5.4. SUMMARY OF THE ANALYSES

Outcomes	Populations	Measures of effect and Models	Sub-Group analyses
Primary outcome and components *(outcomes 1, 1a, 1b)*	ITT	RR GLM Adjusted only for randomisation strata	By gestation strata
			By FiO_2 level at study entry
			By region
		RR GLM Adjusted*	By gestation strata
	As-Treated	RR GLM Adjusted only for randomisation strata	By gestation strata
	PP	RR GLM Adjusted only for randomisation strata	By gestation strata
Key clinical and safety outcomes *(outcomes 2-7)*	ITT	RR GLM Adjusted only for randomisation strata	By gestation strata
			By FiO_2 level at study entry
Binary secondary outcomes *(outcomes 9, 22-29)*	ITT	RR GLM Adjusted only for randomisation strata	By gestation strata
Continuous secondary outcomes *(outcome 28)*	ITT	Mean difference GLM Adjusted only for randomisation strata	By gestation strata
Skewed continuous secondary outcomes *(outcomes 8, 23-27)*	ITT	Median difference Quantile regression Adjusted only for randomisation strata	By gestation strata
MIST intervention procedural and safety outcomes *(outcomes 30-52)*	As-Treated	Descriptive Statistics	By gestation strata

**adjusted for gestational age stratum, birth weight <10th percentile, sex, mode of delivery, plurality, antenatal glucocorticoid exposure, 5-minute Apgar score.*

Signature of Principal Investigator:  Date 30-10-2020

Print Name Prof Peter Dargaville

Signature of Trial Statistician:  Date 30-10-2020

Print Name Prof John Carlin

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