Multicentre Randomised Controlled Trial of Surfactant Plus Budesonide to Improve Survival Free of Bronchopulmonary Dysplasia in Extremely Preterm Infants

The PLUSS TRIAL

Preventing Lung Disease Using Surfactant + Steroid

Trial Sponsor: Melbourne Children’s Trials Centre, Murdoch Childrens Research Institute (MCRI), Melbourne, Australia

Funding: National Health and Medical Research Council (NHMRC), Australia Program Grant (No. 1113902)

Trial Registration: Australian New Zealand Clinical Trials Registry; ACTRN12617000322336

Version 8.1, 4 September 2020
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<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>AE</td>
<td>Adverse Event</td>
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<tr>
<td>ANZNN</td>
<td>Australian and New Zealand Neonatal Network</td>
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<tr>
<td>BPD</td>
<td>Bronchopulmonary dysplasia</td>
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<tr>
<td>CEBU</td>
<td>Clinical Epidemiology and Biostatistics Unit</td>
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<tr>
<td>CPAP</td>
<td>Continuous positive airway pressure</td>
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<tr>
<td>CRF</td>
<td>Case report form</td>
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<td>DSMB</td>
<td>Data and Safety Monitoring Board</td>
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<tr>
<td>FiO₂</td>
<td>Fraction of inspired oxygen</td>
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<tr>
<td>HF</td>
<td>High-flow</td>
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<tr>
<td>HREC</td>
<td>Human Research and Ethics Committee</td>
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<tr>
<td>ICS</td>
<td>Inhaled corticosteroids</td>
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<td>IRB</td>
<td>Institutional Review Board</td>
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<tr>
<td>LISA</td>
<td>Less invasive surfactant administration</td>
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<td>LOS</td>
<td>Late-onset sepsis</td>
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<tr>
<td>MIST</td>
<td>Minimally invasive surfactant administration</td>
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<tr>
<td>NHMRC</td>
<td>National Health and Medical Research Council, Australia</td>
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<tr>
<td>NICU</td>
<td>Neonatal intensive care unit</td>
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<tr>
<td>NICHD</td>
<td>National Institutes of Child Health and Human Development</td>
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<tr>
<td>NIPPV</td>
<td>Non-invasive positive pressure ventilation</td>
</tr>
<tr>
<td>PDA</td>
<td>Patent ductus arteriosus</td>
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<tr>
<td>PICF</td>
<td>Participant Information and Consent Form</td>
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<tr>
<td>PMA</td>
<td>Post-menstrual age</td>
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<tr>
<td>RDS</td>
<td>Respiratory distress syndrome</td>
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<tr>
<td>RWH</td>
<td>The Royal Women’s Hospital, Melbourne, Australia</td>
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<tr>
<td>SAE</td>
<td>Serious adverse event</td>
</tr>
<tr>
<td>SpO₂</td>
<td>Peripheral oxygen saturation</td>
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<tr>
<td>SSI</td>
<td>Significant safety issue</td>
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<tr>
<td>SUSAR</td>
<td>Suspected unexpected serious adverse reaction</td>
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<tr>
<td>TGA</td>
<td>Therapeutic Goods Association</td>
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<tr>
<td>USM</td>
<td>Urgent safety measure</td>
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TRIAL SUMMARY

RESEARCH QUESTION

Does administration of intra-tracheal budesonide during the early treatment of respiratory distress syndrome (RDS) in extremely preterm infants increase survival without bronchopulmonary dysplasia (BPD) at 36 weeks’ postmenstrual age (PMA)?

BACKGROUND

Bronchopulmonary dysplasia (BPD) is a chronic inflammatory lung disease characterised by disordered alveolar and vascular development, most commonly affecting extremely preterm infants exposed to mechanical ventilation and oxygen therapy for RDS. BPD is associated with mortality, and adverse long-term pulmonary and neurodevelopmental outcomes. Despite advances in neonatal care including antenatal corticosteroids, exogenous surfactant, and the increasing use of non-invasive respiratory support, the incidence of BPD has increased in the state of Victoria in 2005 compared with earlier eras; e.g., from 43% in 1997, to 56% in 2005 (adjusted odds ratio 2.67, 95% confidence interval 1.60, 4.46; P<0.001).

HYPOTHESIS

Early administration of intra-tracheal budesonide combined with exogenous surfactant as the carrying vehicle, to extremely preterm infants will increase survival free of BPD at 36 weeks’ PMA.

STUDY DESIGN

A multicentre, two-arm, parallel, double-blind, randomised controlled trial.

STUDY POPULATION

Extremely preterm infants 22-27 weeks’ completed gestation admitted to a participating neonatal intensive care unit (NICU), who fulfil the entry criteria detailed below.

RECRUITMENT

Inclusion Criteria

All of the below are required for an infant to be eligible:

1. Less than 48 hours of age
2. Receiving mechanical ventilation via an endotracheal tube, or infants receiving non-invasive respiratory support including continuous positive airway pressure (CPAP), non-invasive intermittent positive pressure ventilation (NIPPV) or nasal high flow (HF), and a clinical decision to treat the infant with exogenous surfactant.
Exclusion Criteria

1. Prior treatment with corticosteroids for the prevention of lung disease (inhaled, nebulised, intra-tracheal, or systemic)
2. Infant is considered non-viable or is not going to be admitted to the neonatal intensive care unit (NICU)
3. Known or suspected major congenital anomaly that is likely to affect respiratory status (eg. upper airway obstruction congenital lung malformation, major congenital heart disease); or severe pulmonary hypoplasia following premature rupture of fetal membranes with resultant severe oligo/anhydramnios, where the clinical, based on clinical assessment on the first postnatal day, believes survival is unlikely.
4. Infant likely to be transferred to another non-participating NICU within 24 hours of birth

RANDOMISATION

A computerised random number generator will be used to select random permuted blocks with at least three different block sizes. Randomisation with balanced variable block sizes will be used, stratified by study centre, gestational age (22-25 weeks’ vs. 26-27 weeks’ completed gestation), prior surfactant therapy, and mode of respiratory support at randomisation (mechanical ventilation via an endotracheal tube vs. non-invasive respiratory support). After prospective, written informed parental/guardian consent, a web-based computerised system designed and administered by the Murdoch Children’s Research Institute will be accessed by computer to ascertain each infant’s allocation. Enrolled infants will be randomised in a 1:1 ratio to surfactant plus budesonide (intervention), or surfactant alone (control). Eligible infants from a multiple pregnancy will be randomised individually.

STUDY INTERVENTION

Treating clinicians, investigators and outcome assessors will be blinded to allocation. Infants randomised to the intervention arm will receive budesonide 0.25 mg/kg mixed with surfactant (Curosurf®, Chiesi Farmaceutici, Parma, Italy). Infants randomised to the control arm will receive surfactant alone. Once enrolled, infants will receive at least one, and no more than two, doses of surfactant +/- budesonide. For infants receiving non-invasive respiratory support, the allocated treatment may be given by any method that permits direct instillation into the trachea.

MANAGEMENT OF INFANTS IN NICU

Inhaled or nebulised corticosteroid treatment will not be permitted in the trial at any time. All other management of enrolled infants after the intervention will be at the discretion of the clinical team. This includes escalating and weaning respiratory support, prescribing systemic (oral or intravenous) postnatal corticosteroids, oxygen saturation targeting, antibiotics for late-onset sepsis (LOS), enteral feeding, and diagnosis and treatment of a patent ductus arteriosus (PDA). Caffeine therapy is expected to be universal in this population. Oxygen saturation targets will be based on local unit guidelines. Participation in other clinical studies will be permitted pending formal notification to, and approval by, the PLUSS Steering Committee.
OUTCOMES

Primary Outcome

Survival free of physiological BPD at 36 weeks’ PMA.

Secondary Outcomes

Include: the rate of death prior to 36 weeks’ PMA, BPD at 36 weeks’ PMA, death before hospital discharge, respiratory status at 40 weeks’ PMA, other major neonatal morbidities (intraventricular haemorrhage, periventricular leukomalacia, retinopathy of prematurity, necrotizing enterocolitis, spontaneous intestinal perforation, pneumothorax requiring drainage, PDA treated pharmacologically or with surgery, LOS, duration of respiratory support and supplemental oxygen therapy, length of hospital stay, cost of hospitalisation, cost-effectiveness analysis, neurodevelopmental and medical assessment at 2 years’ corrected age.

SAMPLE SIZE

With a sample size of 1038 infants (519 in each group), the study has 90% power to detect a relative reduction of 20% in death or BPD at 36 weeks’ corrected gestation, from the anticipated event rate of 50% in the control arm to 40% in the intervention (budesonide) arm, alpha error 0.05. To allow for 2% study withdrawals or losses to follow-up, we aim to recruit 1060 infants (530 in each arm).

TRIAL PLAN

The PLUSS Trial will commence at The Royal Women’s Hospital, Melbourne, in 2018, with a progressive roll-out to other Australasian and selected international centres during 2018-19. With 12-15 participating centres recruiting 60% of eligible infants, we estimate that recruitment of patients will take 4.5 years with another six months to completion allowing time for assessment of all outcomes and analysis.
1 BACKGROUND

1.1 Introduction
Despite advances in neonatal care, bronchopulmonary dysplasia (BPD) continues to make a prominent contribution to adverse health outcomes for infants born extremely preterm (<28 weeks’ gestation). BPD was originally described by Northway in 1967, as a radiological, pathological and clinical pattern of lung injury in moderately preterm infants characterized by pulmonary fibrosis resulting from oxygen and mechanical ventilation. This was the predominant pathophysiology underlying BPD for infants with respiratory distress syndrome (RDS) in the pre-surfactant era. Following the introduction of antenatal steroids for women at risk of preterm birth and exogenous surfactant as standard care for RDS, the rates of BPD have remained static implying a different underlying pathophysiological process. The “New BPD” is characterised by inhibition of angiogenesis and lung alveolar development. Animal models suggest that contributory factors include surfactant deficiency, infection, oxygen toxicity and mechanical ventilation. Although BPD is common in extremely preterm infants, some do not develop BPD, suggesting that it is a preventable disease.

1.2 Burden of Disease
BPD, a chronic lung disease of the preterm infant, is identified by the need for supplemental oxygen and/or respiratory support at 36 weeks PMA. In 2013, almost 600 Australasian infants were diagnosed with BPD after preterm birth at <28 weeks’ gestation. In the USA, an estimated 10,000 infants each year are diagnosed with BPD, defined as a supplemental oxygen requirement at 36 weeks’ corrected gestation. A Vermont Oxford Network study of over 400,000 infants in the USA showed that, in contrast to other important neonatal morbidities, the rate of BPD remained almost static over the last decade: 32% in 2005 to 28% in 2014. Recent large interventional RCTs enrolling extremely preterm infants (born <28 weeks’ gestation) report a BPD rate of up to 50% in those who survive. Moreover, the rate of BPD in this cohort appears to be rising, increasing from 43% in 1997 to 56% in 2005 in infants born <28 weeks.

A diagnosis of BPD confers lasting ill effects on respiratory health and overall wellbeing. Infants with BPD are more likely to be readmitted to hospital with respiratory deterioration in the first year of life, and are more likely to die in infancy than very preterm infants without BPD. Survivors with BPD have an increased risk of neurodevelopmental impairment and their respiratory function remains compromised into adolescence. Emerging evidence indicates that early life origins of adult chronic obstructive lung disease include a genetic predisposition, prenatal exposure to nicotine and prematurity. Forced Expiratory Volume in one minute (FEV₁) as a marker for obstructive lung disease, when compared with term-born term infants, is lower for both surviving preterm-born children with and without BPD. The effects on expired airflow appear to be worsening, with poorer FEV₁ values at 8 years of age in the 2005 extremely preterm cohort than the 1997 cohort (VICS data) which is ominous for respiratory health.

Reducing the burden of BPD by optimising the respiratory care for extremely preterm infants and interrupting the inflammatory pathway by using a targeted intervention modifying the pathogenic pathway, may yield long term pulmonary benefits extending to adulthood.
1.3 Pathogenesis of BPD – a target for corticosteroids

Laboratory and clinical studies suggest a crucial role for lung inflammation (pre- and post-natal) and host immune response in the pathogenesis of BPD.\(^4\)\(^2\)\(^1\) The transcription factor nuclear factor B (NF-B) is a central (cytoplasmic) cellular mediator of inflammation and linked to the pathogenesis of many inflammatory pulmonary diseases including acute respiratory distress syndrome, asthma, and chronic obstructive pulmonary disease.\(^2\)\(^2\) NF-B regulates the cellular response to inflammation, oxidant stress, airway stretch from mechanical ventilation. Agents aimed at modulating this central pathway of inhibiting NF-B activation which may prevent BPD have been studied and include dexamethasone, azithromycin, nitric oxide, and pentoxifylline.\(^2\)\(^2\)\(^-\)\(^2\)\(^4\)

![Figure 1. Windows of opportunity to prevent BPD. From the National Heart, Lung, and Blood Institute Workshop 2014]({})

A subcommittee of perinatal experts of the National Health, Lung, Blood Institute (NHBLI) summarised the current state of knowledge regarding BPD and identified potential points in its pathogenesis susceptible to therapeutic interventions (Figure 1). One recommendation was to study the use of selective anti-inflammatory therapies, and novel modulators of innate immunity to attenuate the inflammatory response in the developing lung to potentially prevent BPD.\(^5\)\(^\)\(^7\)

1.4 Steroids to interrupt the pathogenesis of BPD

The history of corticosteroid therapy to prevent or treat BPD in preterm infants highlights the paramount importance of well-designed clinical trials incorporating long-term outcomes before adopting novel therapies. As inflammation is a primary mediator of injury in the pathogenesis of BPD, anti-inflammatory agents such as postnatal corticosteroids have long been the focus of preventive interventions. Whilst systemic corticosteroids reduce inflammation and improve respiratory function, their early use may be associated with many side effects.
The short term adverse effects of postnatal systemic (oral or intravenous) corticosteroids in preterm infants include hyperglycaemia, sepsis, hypertension, hypertrophic obstructive cardiomyopathy, gastro-intestinal haemorrhage, spontaneous intestinal perforation, growth suppression and hypothalamic pituitary adrenal axis suppression\textsuperscript{25}. Long term adverse effects include neurodevelopmental delay and cerebral palsy\textsuperscript{16}. Current recommendations from the American Academy of Pediatrics (AAP) state that high doses (>0.5mg/kg dexamethasone) of systemic corticosteroids and early (<8 days of age) exposure should be avoided.\textsuperscript{26} Clinicians are advised to use systemic corticosteroids with caution, balancing the potential adverse effects of treatment with the risk of BPD. The AAP suggests that further randomised controlled trials (RCTs) are warranted to optimise treatment and outcomes for infants at risk of BPD.\textsuperscript{26}

Given the potential serious adverse effects of high-dose, early systemic corticosteroid administration, safer alternatives are sought. Recently, the French PREMILOC trial of early, low-dose hydrocortisone (0.5 mg/kg twice per day for 7 days followed by 0.5 mg/kg/day for 3 days, starting in the first 24 hours of age) in 523 extremely preterm infants born 24-28 weeks’ gestation showed an increase in survival without BPD in the hydrocortisone group: from 51% to 60% (adjusted OR 1.48, 95% CI 1.02–2.16, P=0.04).\textsuperscript{27} Rates of death alone and BPD alone were slightly lower in the hydrocortisone group, without reaching statistical significance. Subgroup analyses found a higher rate of sepsis in infants born at 24–25 weeks’ gestation who were treated with hydrocortisone (40% vs. 23%, sub-hazard ratio 1.87, 95% CI 1.09–3.21, P=0.02). Other potential adverse events did not differ significantly between groups. Long-term follow-up to between 21 and 23 months corrected age of 93% of survivors, found no differences in rates of neurodevelopmental impairment or cerebral palsy, or in the mean global developmental quotient score.\textsuperscript{28} There were also no differences in long-term respiratory health or growth.

Whilst the use of low-dose systemic corticosteroid (hydrocortisone) is promising, local administration of corticosteroids to the lung may be optimal: this strategy has the potential to maximise anti-inflammatory effects on the distal airway, minimise systemic absorption, and decrease the risk of adverse effects.

1.5 Inhaled and nebulised corticosteroids (ICS)

Inhaled medications such as beta-agonists, anticholinergics and corticosteroids have been used to treat airway disease in infants with established or evolving BPD. Although ß-agonists acutely improve lung function\textsuperscript{29} and ICS improve lung compliance and reduce pulmonary resistance\textsuperscript{30}, evidence for clinical benefit is not strong. This may be due to the heterogeneous treatment response, which in turn may be related to varying modes of administration and difficulties in delivery of the active drug to the distal airway\textsuperscript{31}, particularly in preterm infants receiving respiratory support.

Administering ICS via a spacer or nebulizer could avoid the adverse effects of systemic corticosteroids and provide anti-inflammatory benefits to the preterm infant recovering from RDS, but the results from studies of this intervention are conflicting. In a study of ICS (beclomethasone), Giep and colleagues found no difference between plasma cortisol levels in the treatment or control groups, suggesting no adrenal suppression from systemic distribution.\textsuperscript{32} However, delivering inhaled and nebulised medications to extremely preterm infants is technically challenging and early trials did not show benefit.\textsuperscript{33,34} Cole and colleagues found that ICS (beclomethasone) led to earlier extubation and less systemic corticosteroid use, but compared with placebo, no reduction in BPD.\textsuperscript{34}
The NEUROSIS trial\textsuperscript{10} was a large study of 863 extremely preterm infants comparing early prolonged ICS (budesonide) with placebo. The interventions were administered on the first day after birth until infants no longer required oxygen and positive-pressure support or until 32 weeks’ corrected gestation. In the budesonide group, there was a borderline reduction in the combined outcome of BPD or death from 46\% to 40\% (P=0.05). BPD alone was reduced from 38\% to 28\% (P=0.004), however there was a concerning, although statistically non-significant increase in mortality from 14\% to 17\% (P=0.17) in the budesonide group.

A recent meta-analysis combining data from six small trials of ICS and data from the NEUROSIS trial, reported a significant reduction in BPD (RR=0.77, 95\% CI 0.65 to 0.91, n =1168).\textsuperscript{15} These studies were heterogeneous in their inclusion criteria; some trials enrolled infants at risk of BPD, and others infants with already-evolving BPD. However, the relatively high daily dose of 0.8 mg/kg and long duration of treatment (4-6 weeks in the NEUROSIS trial) highlight the difficulty of delivering ICS to the distal airway and raise concerns about systemic absorption, which was not assessed in this study.

\subsection*{1.6 Intra-tracheal corticosteroids using surfactant as a carrier}

Use of surfactant as a carrier vehicle may improve delivery and effectiveness of intra-tracheal budesonide and increase survival without BPD in extremely preterm infants

The mixing of corticosteroids or antibiotics with surfactant results in a rapid and far more uniform distribution of the active drug to the distal airspaces after tracheal instillation.\textsuperscript{36,37} The use of surfactant as a vehicle to distribute corticosteroid has shown short term benefits and effectiveness as an intervention in animal models.\textsuperscript{38-41} Nimmo et al showed that, within a specific concentration ratio, the addition of dexamethasone to bovine surfactant extract did not alter the surface properties of the surfactant, and, after instillation to rats, was well distributed throughout the lung.\textsuperscript{40} The study found greater efficacy in the delivery of the steroid using surfactant as the vehicle for distribution compared to saline. Chen and colleagues found combined administration of intra-tracheal surfactant and corticosteroid improved gas exchange, and ameliorated markers of acute lung inflammation with less histopathological lung injury.\textsuperscript{41} Using budesonide mixed with bovine surfactant, Yang and colleagues demonstrated no loss of surfactant activity in an animal model of RDS compared to delivering surfactant alone.\textsuperscript{39} Chiesi Farmaceutici (Parma, Italy) have conducted both \textit{in vitro} and \textit{in vivo} (rabbit pup with RDS) studies investigating the stability of, and surfactant activity, following mixing of budesonide with Curosurf. These studies show no loss of surfactant activity \textit{(in vitro} using captive bubble surfactometry\textit{)} and clinical improvements (in oxygenation) that mirror Curosurf use alone in the rabbit pup with RDS using the doses of budesonide (0.25mg/kg) as described by Yeh and colleagues\textsuperscript{42,43} \textit{(personal communication, Chiesi Farmaceutici, October 2016)}

In a small single centre pilot study, Yeh and colleagues showed that intra-tracheal administration of budesonide using surfactant as a vehicle to distribute it to the lungs was feasible.\textsuperscript{42} The group then undertook a multicentre, randomised trial in 265 infants with birth weight <1500g and severe RDS requiring mechanical ventilation and a fraction of inspired oxygen (FiO\textsubscript{2}) >0.50 within 4 hours of birth.\textsuperscript{43} Infants were randomly assigned to bovine surfactant alone (100 mg/kg) or surfactant and budesonide (0.25 mg/kg) every eight hours until each infant had an FiO\textsubscript{2} <0.30 or was extubated from mechanical ventilation. Although the protocol allowed infants to receive a maximum of 6 doses of the intervention, most received 1-2 doses. The surfactant plus budesonide group had a
significantly lower incidence of the combined outcome of BPD or death, 42% vs. 66% (P<0.001). There were no adverse effects of budesonide detected.

In the study of Yeh et al\(^4\), the mean gestation of enrolled infants mechanically ventilated with severe BPD was 26 weeks. Whilst this is a recognisable group of infants, more infants are being managed using non-invasive respiratory support to avoid or limit the duration of mechanical ventilation, and it is important to know whether budesonide has similar beneficial effects in infants at high risk of BPD irrespective of the primary mode of respiratory support. The large treatment effect seen in the study has been viewed with some scepticism by clinicians, and needs to be reproduced in a larger, more pragmatic trial. Given the importance of survival free of BPD as an outcome, clinicians would accept a much smaller increase in its incidence. In addition, the bovine surfactant (Survanta™) used in Yeh’s study requires a larger volume, and is less commonly prescribed globally to treat neonatal RDS, as there is a preference to use porcine derived (lower volume) surfactant, further limiting the applicability of their results. Nevertheless, the results of the study are promising, with the findings suggesting an enhanced budesonide delivery system to the distal lung compared with nebulised or inhaled preparations.

No studies of this type have yet been conducted using porcine surfactant (poractant alfa, Curosurf, Chiesi Farmaceutici, Parma, Italy) to deliver the corticosteroid, or in infants receiving non-invasive respiratory support. Curosurf requires a lower volume than other surfactants, and is the most commonly prescribed animal-derived surfactant in Australasia.\(^6\) PLUSS will be the first study to determine if budesonide mixed with Curosurf and instilled into the tracheal improves survival without BPD. This study will also be unique in including infants on non-invasive respiratory support, where the lower dosing volume of Curosurf affords the possibility of using a minimally-invasive technique of administration, e.g. via a thin catheter.

1.7 **Significance of the study**

Exogenous surfactant is a proven effective therapy for RDS in preterm infants. Combining budesonide with surfactant is a simple intervention that may prevent BPD in the high-risk population of extremely preterm infants. A previous study of budesonide administration admixed with surfactant in mechanically ventilated infants provides preliminary evidence of feasibility, safety, and potential benefit of administering corticosteroids directly into the lung via the intra-tracheal route during the acute phase of neonatal RDS.\(^4\)

PLUSS will be the definitive study examining whether early intra-tracheal corticosteroid improves survival without BPD in extremely preterm infants. Its pragmatic and inclusive design, targeting all extremely preterm infants with RDS, regardless of their initial mode of respiratory support, will mean the results are applicable worldwide. Should intra-tracheal budesonide reduce BPD, without adverse effects, this readily available and inexpensive intervention could be introduced immediately into routine clinical practice. This intervention, may then have the potential to modify lung injury and translate into significant respiratory and general health benefits in later childhood and adulthood.
2 TRIAL PROTOCOL

2.1 Aim
To evaluate the safety and efficacy of early intra-tracheal corticosteroid (budesonide) in extremely preterm infants with respiratory distress syndrome.

2.2 Hypothesis
Early administration of intra-tracheal budesonide using exogenous surfactant as a carrying vehicle to extremely preterm infants born <28 weeks' completed gestation will increase survival free of physiological BPD at 36 weeks' PMA.

2.3 Study design
A multicentre, two-arm, parallel, double-blind, randomised controlled trial. Families, healthcare providers, outcome assessors and data analysts will be blinded to the randomisation group.

2.4 Study population
Extremely preterm infants 22-27 weeks' completed gestation admitted to a participating neonatal intensive care unit (NICU), who fulfil the entry criteria detailed below.

2.5 Recruitment

2.5.1 Inclusion criteria
All of the below are required for an infant to be eligible:

1. Less than 48 hours of age
2. Receiving mechanical ventilation via an endotracheal tube or non-invasive respiratory support including CPAP, NIPPV or nasal high flow, and a clinical decision to treat the infant with exogenous surfactant (first or subsequent dose)

2.5.2 Exclusion criteria
Any of the below make an infant ineligible:

1. Prior treatment with corticosteroids for the prevention of lung disease (inhaled, nebulised, intra-tracheal, or systemic)
2. Infant is considered non-viable or is not going to be admitted to intensive care
3. Known or suspected major congenital anomaly that is likely to affect respiratory status (e.g., upper airway obstruction, congenital lung malformation, major congenital heart disease); or severe pulmonary hypoplasia following premature rupture of fetal membranes with resultant severe oligo. Anhydramnios, where the clinician, based on clinical assessment on the first postnatal day, feels survival is unlikely.
4. Infant likely to be transferred to another non-participating NICU within 24 hours of birth

A record of all infants screened for eligibility but not enrolled will be maintained.

2.6 Consent
Informed parental/guardian consent will be obtained prior to randomisation by a researcher or clinician trained to obtain consent for the trial. Consent will be obtained either antenatally or...
postnatally. In all cases, written consent will be obtained using a specifically designed Participant Information and Consent Form (PICF) which may be modified to meet the requirements of each participating centre’s human research ethics committee (HREC). A copy of the PICF will be provided to the parents and also documented in the infant’s hospital record and trial Case Report Form (CRF).

Parent(s)/guardian(s) are free to withdraw their infant from the study at any time. Permission will be sought from families who withdraw their infant from the study to allow researchers to continue data collection from their child’s hospital record. The reason(s) for withdrawal from the study will be recorded in the CRF and included in the final report.

In all Australian centres, separate consent will be obtained for Medicare Linkage using Department of Health consent forms to enable follow up of study participants’ use of pharmaceuticals and medical services until 2 years corrected age. In other countries, consent will be sought for equivalent data.

2.7 Randomisation
When eligibility of an infant is confirmed, and prospective consent obtained, the infant will be assigned to either receive surfactant plus budesonide, or surfactant alone, using a web-based randomisation system with an allocation ratio of 1:1. A checklist on the website will be used to confirm eligibility prior to randomisation. Infants will remain in their allocated group for repeat interventions (if applicable), with a maximum of two study interventions (Figure 2). The randomisation schedule will be provided by the Clinical Epidemiology and Biostatistics Unit (CEBU) at the Murdoch Children’s Research Institute, Melbourne, Australia.

Randomisation with balanced variable block sizes will be used, stratified by study centre, gestational age (22-25 weeks’ vs. 26-27 weeks’ completed gestation), prior surfactant therapy, and mode of respiratory support at randomisation (mechanical ventilation via an endotracheal tube vs. non-invasive respiratory support). Multiple births where more than one infant is eligible will be randomised individually. Eligibility will be confirmed before the clinical or research team will be able to randomise an infant. A sealed opaque envelope will be identified by the unique study ID generated from the web based server.

2.8 Blinding
Families, healthcare providers, outcome assessors and data analysts will be blinded to randomisation groups.

Although difficult to distinguish, there are subtle differences in the appearance and volume of the study drugs in the intervention and control arms. To maintain blinding, the study drugs will be prepared by an intervention team (Section 2.9.2) whose members are not directly involved in the clinical care of the infant, and not involved in data collection or outcome assessments for the study.

The pharmacy departments of each participating centre and CEBU will be the only other personnel aware of the allocated study intervention; they will also not be involved in data collection or outcome assessments for the study. Pharmacies will maintain a logbook of allocated study drugs and doses.
Neither the PLUSS Steering Committee nor site researchers will be aware of the allocated interventions, and will not be permitted access to these data until trial completion.

2.8.1 Breaking of the study blind
The randomisation code for an individual participant may only be unblinded in emergency situations, where the Site Principal Investigator decides a participant cannot be adequately treated without knowing the identity of their treatment allocation or in circumstances where this information is deemed necessary for reporting a suspected unexpected serious adverse reaction (SUSAR). To break the randomisation code, the Site Principal Investigator/delegate should contact the PLUSS Trial Coordinating Centre. Efforts should be made to restrict the number of research team members made aware of the treatment allocation.

2.9 Study intervention

2.9.1 Setting
The study intervention will be prepared in the NICU of participating centres, and will be administered in either the delivery room or the NICU to enrolled infants.

2.9.2 Preparation of the study intervention
A two-person intervention team will be convened from staff on-duty who are not currently involved in the clinical care of the infant. Intervention teams will be available 24-hours and will consist of either: a nurse and a doctor, two nurses, or a nurse and a pharmacist. The makeup of the intervention team may vary with the timing of the intervention (in-hours it is more likely that a pharmacist will be available). No members of the intervention team will be involved in data collection or outcome assessments for the study.

The study drugs will be made available in the NICU. The intervention will be prepared in an area away from the infant and clinical team, where budesonide and surfactant (Curosurf) are available. The intervention team will open the randomisation envelope and identify a further two sealed mini envelopes labelled “Study ID: XX-X-XXX, Intervention 1” and “Study ID: XX-X-XXX, Intervention 2”. The intervention team will open the first (Intervention 1) envelope and draw up the correct dose (to one decimal place) of Curosurf in a 3 mL (for infants with birth weight <1000 g) or 5 mL (for infants with birth weight ≥1000 g) syringe. For infants allocated to Curosurf plus budesonide, the dose of budesonide (to two decimal places) will be drawn up in a separate 1 mL syringe, then added to the syringe containing the Curosurf. To further maintain blinding, an opaque trial label will be applied around the syringe in order to hide the volume and appearance of the contents from clinicians whilst it is being administered. The Curosurf dose and the PLUSS Study ID will be charted on the infant’s prescription chart or electronic medical record for each intervention.

A nurse or doctor from the intervention team will then go to the bedside of the enrolled infant. Before the allocated treatment is administered, pre-intervention patient observations will be documented in the CRF by the clinical or research team. Immediately prior to administration the syringe will be hand-mixed by inverting it several times. The intervention team nurse or doctor will then either administer the prepared treatment when directed by the clinical team, or hand the syringe to the clinical team to administer (depending on local administration protocols). The second mini envelope from the randomisation envelope will be stored in the infant’s Study Pack, to be opened by the intervention team for the second intervention 6-12 hours later (if applicable).
In this pragmatic study, the following methods of intra-tracheal instillation will be permitted: standard bolus administration through an endotracheal tube (ETT) that will remain in situ with ongoing mechanical ventilation, INSURE (intubate, surfactant, extubate) technique via an ETT, or using a minimally-invasive (MIST) or less-invasive (LISA) method in those infants receiving non-invasive respiratory support (CPAP, NIPPV or HF). \(^4^4\)

Budesonide will be distributed to the NICU by the hospital pharmacy department and stored at room temperature. The Curosurf used in the study will be accessed from ward stock. Stores of Curosurf and budesonide will be maintained by the NICU pharmacist. Expiry dates for all medications will be clearly labelled and the pharmacist will be responsible for removing expired medications and replenishing stock.

Budesonide dosing for the PLUSS trial will be based on an earlier study by Yeh and colleagues.\(^4^3\)

- Curosurf dose (both arms) 200mg/kg initial dose; subsequent dose 100mg/kg (if applicable)
- Budesonide dose (intervention arm) 0.25mg/kg (0.5 mL/kg of a 1mg/2mL solution)

A maximum of two study interventions will be permitted in the trial. Therefore, if an infant was enrolled after receiving an initial dose of surfactant, the maximum number of surfactant doses that an infant may receive as part of the trial protocol is three (Figure 2).

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**Figure 2.** PLUSS Study Participant Flow Chart.
2.10 Post-randomisation management of infants in NICU
Inhaled or nebulised corticosteroid treatment will not be permitted in the trial. All other management of enrolled infants after randomisation will be at the discretion of the clinical team. This includes escalating and weaning respiratory support, prescribing systemic (oral or intravenous) postnatal corticosteroids, oxygen saturation targeting, antibiotics for late-onset sepsis (LOS), enteral feeding, and diagnosis and treatment of a patent ductus arteriosus (PDA). Caffeine therapy is expected to be universal in this population. Oxygen saturation targets will be based on local unit guidelines.

2.11 Assessment of BPD at 36 weeks’ PMA
The reported incidence of BPD varies due to inconsistent definitions and the inherent variability amongst NICUs in their approach to oxygen therapy and oxygen saturation targets. Given the importance of BPD as the primary outcome in the PLUSS Trial, a standardised approach to the diagnosis of BPD has been incorporated into the trial design.

Infants will be assessed for BPD between 36\(_{+0}\) and 36\(_{+6}\) weeks PMA. Based on the National Institute of Child Health and Human Development (NICHD) consensus statement and definition of “physiological BPD”\(^45\), a ‘Modified Walsh’ test will be used in this study to define BPD.\(^46\) In addition, assuming a degree of ventilation-perfusion mismatch in the lungs of infants with physiological BPD, a “Shift test” will be applied\(^47\). These assessments (Section 2.9.5) are currently being used across all centres within the Australian and New Zealand Neonatal Network (ANZNN).

2.11.1 Shift Test and Modified Walsh Air Reduction Trial

Shift Test
1. For infants receiving mechanical ventilation via an endotracheal tube, CPAP, NIPPV, or HF, the FiO\(_2\) will be adjusted to maintain the peripheral oxygen saturation (SpO\(_2\)) between 90 - 94% over a 15-minute period. Every minute the FiO\(_2\) and SpO\(_2\) will be noted and modal values reported.

2. For infants on no respiratory support or oxygen supplementation, the SpO\(_2\) will be recorded at 1-minute intervals for 15 minutes.

3. Infants receiving ‘low flow’ oxygen therapy will have their supplemental oxygen requirement evaluated by the use of incubator oxygen. If the average FiO\(_2\) required to maintain SpO\(_2\) 90-94% is <0.3, the infant will have a further assessment (“Modified Walsh Air Reduction Trial)\(^46\) to determine whether they are able to maintain their SpO\(_2\) in air.

Modified Walsh Air Reduction Trial
For all infants receiving supplemental oxygen but an effective FiO\(_2\) <0.3* a Modified Walsh Air Reduction Trial will be applied. These infants will have stepwise FiO\(_2\) reductions five minutes apart until either room air (21% oxygen) is being administered, or the SpO\(_2\) is no longer in the target range for the participating unit. A successful room air trial will be defined as SpO\(_2\) readings in the target range for 30 minutes in room air, with any nasal prongs removed.

*Effective FiO\(_2\) can be determined using an online calculator: https://urresearch.rochester.edu/fileDownloadForInstitutionalItem.action;jsessionid=C0030541446256DA6DFC72B2E104F9EC?itemId=2913&itemFileId=4079
The Shift test takes around 20 minutes to complete; the Modified Walsh Air Reduction Trial takes another 30 minutes if required.

2.12 Data collection

2.12.1 Data management
The PLUSS investigators at each site will be responsible for the collection of data which will be sourced from the medical notes of the mother and infant, parents, clinical staff, and bedside clinical charts. Paper CRFs and/or electronic data capture systems such as tablets or laptops at the patient’s cot-side will be used, and data will be entered into an electronic database (REDCap™, Vanderbilt University) that will be designed and managed through CEBU. Completed CRFs will be checked for completeness and accuracy by researchers against the source data. All data will be securely stored for 25 years, and then securely destroyed/deleted.

2.12.2 Study data for enrolled infants
Data collection will include the outcomes described below (Section 2.12), as well as the following screening and baseline data:

a) Eligibility and randomisation data: Confirmed eligibility (meets all of the inclusion and none of the exclusion criteria), randomisation date and time, study number
b) Maternal data: age, parity, ethnicity, education level, employment status, family structure (single parent household), exposure to antenatal corticosteroids (including type, dose and completeness), presence of chorioamnionitis (clinical and/or histological diagnosis), duration of rupture of membranes, antibiotics during labour, type of labour, type of anaesthetic, mode of delivery
c) Baseline infant data: date and time of birth, gestational age, sex, birth weight, head circumference, multiplicity, treatments and resuscitation received in the delivery room, Apgar scores at 1 and 5 minutes.
d) Pre-intervention data: blood gas analysis results, most recent blood sugar level, surfactant treatment (including type, method of administration and dose), previous and current respiratory support (type, duration, and settings), fraction of inspired oxygen, antibiotic treatment, caffeine treatment, treatment with medication for a patent ductus arteriosus, treatment for hypotension, presence of a pneumothorax and treatment, episode(s) of hypoglycaemia
e) Intervention data: date and time of intervention(s), clinical condition during and immediately after the intervention (episodes of bradycardia, desaturation, escalation of respiratory support level or resuscitation).

2.13 Planned long term follow-up studies
We recognise that survival free of respiratory disease, normal lung function later in childhood, and longer-term development are more important outcomes than any short-term respiratory outcome determined during the first months after birth. However, we cannot incorporate outcomes beyond 2 years corrected age into the current trial protocol until additional funding is secured. Families will be notified of our intention to conduct longer-term health and development assessments, and will have the opportunity to provide the research team consent in the future, to enable their child’s participation.
2.14 Outcomes

2.14.1 Primary outcome
Rate of survival free of physiological BPD at 36 weeks’ PMA.

Physiological BPD will be assessed between 36\textsuperscript{+0} and 36\textsuperscript{+6} weeks’ PMA. Infants will be defined as having physiological BPD if any of the following criteria are met:

1. Receiving mechanical ventilation via an endotracheal tube, CPAP, NIPPV or HF ≥2L/min
2. An effective FiO\textsubscript{2} ≥0.3 if receiving supplemental oxygen or nasal prongs <2L/min to maintain target oxygen saturations
3. An effective FiO\textsubscript{2} <0.3 if receiving supplemental oxygen or nasal prongs <2L/min to maintain target oxygen saturations AND an unsuccessful air reduction trial

2.14.2 In-hospital secondary outcomes

- Death before 36 weeks’ PMA
  - Primary cause of death listed on death certificate
  - Independent categorisation of death (respiratory vs. non-respiratory)
  - Mode of dying (with/without cardiopulmonary resuscitation, with/without withdrawal or withholding of life-sustaining interventions)

- Survival with physiological BPD at 36 weeks’ PMA
- ‘Clinical BPD’, defined as receiving any supplemental oxygen or any form of respiratory support (mechanical ventilation, CPAP, NIPPV or HF) at both 36 weeks’ and 40 weeks’ (term) PMA
- BPD severity/grade at 36 weeks’ PMA, as defined by Jobe and Bancalari\textsuperscript{45}, and by the National Institute of Child Health and Human Development (USA)\textsuperscript{49}
- Treatment with the following medications:
  - Systemic corticosteroids (including age at treatment, type, dose(s), duration)
  - Vitamin A
  - Bronchodilators
  - Diuretics
  - Caffeine or other methylxanthines

- For infants who die prior to hospital discharge:
  - Mode of and timing of death
  - Cause of death

- Intraventricular haemorrhage on cranial ultrasound (worst grade during admission)
  - All grades (as defined by Papile)\textsuperscript{50,51}
  - ‘Severe’ (grades III or IV)

- Cystic periventricular leukomalacia on cranial ultrasound

- Retinopathy of prematurity (ROP) with maximum stage in each eye, as defined by the International Classification of Retinopathy of Prematurity\textsuperscript{51}
  - ‘Severe’ (stage 2 or above)
  - Treated with laser, cryotherapy or intraocular therapy

- Composite major neonatal morbidity (any of severe intra-ventricular haemorrhage, cystic periventricular leukomalacia, severe retinopathy of prematurity, or physiological BPD) as predictors of long term outcomes\textsuperscript{16}

- Necrotising enterocolitis, Modified Bell’s criteria stage 2 or greater\textsuperscript{52}
• Spontaneous intestinal perforation
• Surfactant dosing:
  o Number of doses
  o Total dose in mg/kg
• Total duration of mechanical ventilation via an ETT (days), and time of first intubation, first extubation, and last day of mechanical ventilation
• Last day of positive pressure respiratory (ETT, CPAP, NIPPV, HF) support (days)
• Last day (postnatal age) of supplemental oxygen (days)
• Discharge home on oxygen
• Length of stay in intensive care (days)
• Length of hospital stay (days)
• Pneumothoraces
  o Any pneumothorax post-randomisation
  o Pneumothorax requiring drainage post-randomisation (needle thoracocentesis or intercostal catheter insertion)
• Pulmonary haemorrhage post-randomisation
  o Clinical diagnosis (eg. blood in aspirate from endotracheal tube)
  o Requiring blood transfusion
• PDA
  o Receiving anti-prostaglandin therapy
  o Receiving surgical ligation
• Any prescribed anti-hypertensive agents during hospitalisation
• Any hyperglycaemia requiring insulin therapy
• Late onset sepsis after 48 hours of age (positive bacterial or fungal culture from a normally sterile site, or negative blood culture but clinical suspicion of sepsis and treatment with antibiotics/antifungals for ≥ 5 days)
• Growth, including weight, length, and head circumference Z-scores, and the change in these Z-scores from birth to 36 weeks’ PMA.53
• Cost-effectiveness analysis performed by a Health Economist

2.14.3 Outcomes related to safety of the intervention
During the 14 days after the first intervention we will note the occurrence of the following outcomes:

• Hyperglycaemia > 10mmol/L, and/or receiving insulin treatment
• Highest systolic and diastolic blood pressure recorded, and whether the infants received antihypertensive medications
• Newly-diagnosed suspected or proven sepsis treated with intravenous antibiotics/antifungals
• Pneumothorax requiring drainage (needle thoracocentesis and/or intercostal catheter insertion)
• Gastrointestinal haemorrhage defined as fresh blood aspirated from an indwelling gastric tube
• Spontaneous intestinal perforation
• Oral candidiasis
• Clinical diagnosis of pulmonary haemorrhage (eg. blood in aspirate from endotracheal tube)
• Severe IVH (grades III or IV)
2.15 Outcomes at 2 years of age (corrected for prematurity)

Families will be asked provide contact details to allow longer-term follow-up to 2 years (corrected for prematurity) and potentially beyond. Contact will be maintained with families of surviving infants by methods such as sending birthday cards and/or trial newsletters that will include a request to update contact details if they are changing.

Around 2 years of age, families will be sent a letter inviting them to attend a follow-up assessment, and that a researcher will be in contact with them by phone and/or email to facilitate this. Although consent is sought at trial entry for access to routine health and education data, separate written informed consent will be obtained prior to any face-to-face assessments at 2 years’ corrected age, and appropriate HREC approval will be sought.

Funding will be sought to also undertake these assessments at all participating centres outside the ANZNN.

Outcomes at 2 years’ corrected age will include:

- Survival free of moderate-severe neurodisability, defined as any one or more of moderate or severe developmental delay, moderate or severe cerebral palsy, deafness or blindness (primary outcome)
- Behaviour
- Physical growth
- Respiratory health
- Parent and patient quality of life
- Household demographics
- Hospital admissions and medication use
- Cost-effectiveness analysis, incorporating admission and medication data of healthcare utilisation during the period between the infant’s discharge from primary hospitalisation and 2 years’ corrected age.

A more detailed methodology for the 2 year assessment (corrected for prematurity) is found in the APPENDIX.

2.16 Statistical analysis and reporting

Data handling, verification and analysis for the PLUSS trial will be performed by CEBU. Statistical analysis will follow standard methods for randomised trials, and reporting of findings will be done in accordance with CONSORT guidelines..

The primary analysis will be by intention-to-treat. For dichotomous outcomes, including the primary outcome, the two treatment groups will be compared using relative risk with 95% CI, both overall, and within the pre-specified subgroups. The individual components of the primary outcome, death or physiological BPD at 36 weeks’ PMA, will be compared between the two treatment groups using relative risk with 95% CI, both overall, and within the pre-specified subgroups. For dichotomous secondary outcomes, the two treatment groups will be compared using relative risk with 95% CI. For continuous outcomes, the two treatment groups will be compared using difference of means, together with 95% CI, for outcome variables which are normally distributed; for outcome variables, which are not normally distributed, the comparison will be difference of medians, with 95% CI.
Analysis of secondary outcomes will not be adjusted for multiple comparisons, but results will be interpreted cautiously.

All comparisons (relative risk, difference of means, difference of medians) will be estimated using regression models with the randomisation stratification factors included as covariates, and with standard errors adjusted to take into account the clustering due to multiple births. We will explore in secondary analysis the potential impact of any imbalance in baseline prognostic factors on the estimate of exposure effect for the primary outcome.

2.16.1 Pre-specified sub-group analyses
For the primary outcome and its components, subgroup analysis will be performed according to the pre-randomisation strata: gestational age, exposure to surfactant prior to randomisation, and mode of respiratory support at randomisation.

In addition, although we acknowledge that the trial is not powered for these analyses, we plan to assess the effect of important factors that might modulate the risk of death and BPD, including sex, small for gestational age, and the presence of chorioamnionitis.

2.17 Economic evaluation
Cost effectiveness analysis will incorporate costs of the intervention and of hospital care (including complications) for the birth admission until death or first discharge home from all health care facilities which includes the primary birth hospital and where appropriate, secondary hospital(s). Cost-effectiveness will be reported as cost per life year gained and cost per infant with BPD prevented for the intervention group compared to control. Extensive one-way and probabilistic sensitivity analyses will be conducted. Family costs associated with the hospital stay will be constructed via systematic review and updated with Australian costs.

2.18 Sample size
From a review of the lead centre (RWH) data and data from recent published studies investigating interventions to reduce death or BPD in extremely preterm infants (NEUROSI S, SUPPORT, BOOST II) the estimated incidence of the composite primary outcome is approximately 50%. With a sample size of 1038 infants (519 in each group), the study has 90% power to detect a relative reduction of 20% in death or BPD at 36 weeks’ PMA, from the anticipated event rate of 50% in the control arm to 40% in the intervention (budesonide) arm, alpha error 0.05. We anticipate approximately 2% study withdrawals or losses to follow-up, so we will aim to recruit 1060 infants (530 in each arm) in order to reach the required final sample size.

2.19 Trial plan
Following institutional research and ethical approval at the lead centre (RWH), the PLUSS trial commenced recruitment in January 2018. We anticipate a total of 5 years of enrolment and data cleaning, analysis and reporting.

2.20 Expected trial timeline

<p>| Mar 2017 | Royal Children’s Hospital HREC (for Australian multi-site approval) |</p>
<table>
<thead>
<tr>
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<th>Event Description</th>
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<td>Jul 2017</td>
<td>HREC submission for New Zealand multi-site approval</td>
</tr>
<tr>
<td>Jan 2018</td>
<td>Commencement of recruitment at RWH</td>
</tr>
<tr>
<td>Jul 2018</td>
<td>Ethics submissions to new centres; trial commencing in Auckland</td>
</tr>
<tr>
<td>Oct 2018</td>
<td>Successful NHMRC Project Grant Funding Announced</td>
</tr>
<tr>
<td>2019-2020</td>
<td>Roll out to other centres in Australia, New Zealand, Canada and Singapore</td>
</tr>
<tr>
<td>Jun 2023</td>
<td>Estimated completion of recruitment, data cleaning and analysis</td>
</tr>
<tr>
<td>Dec 2023</td>
<td>Reporting and dissemination of results</td>
</tr>
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</table>
2.21 Feasibility
We anticipate that with 15-20 participating centres recruiting 50% of eligible infants in this population of 22-27 completed weeks, study recruitment will be completed within five years.

2.22 Data and safety monitoring
An independent Data and Safety Monitoring Board (DSMB) has been established for the PLUSS trial. The roles and responsibilities of the DSMB will be detailed in a separate DSMB Charter. The DSMB includes four independent, experienced neonatologists and a senior statistician. The terms of reference for the DSMB include performance of interim safety analyses, periodic examination of emerging external evidence in relation to adjunctive treatment of RDS with exogenous surfactant and intra-tracheal or ICS, and monitoring of adverse events, deaths, compliance with the trial protocol, and progress of recruitment.

2.22.1 Interim Analyses
The Trial Steering Committee expects that there will be regular safety analyses for the PLUSS trial: after the primary outcome is known for 50, 100, 265 (25% recruitment), 530 (50%), and 800 (75%) infants.

A single interim analysis of the primary outcome and its components will be performed at the midpoint of the trial (primary outcome known for 530 enrolled infants). For this comparison, the statistical approach will be conservative; the DSMB may make a recommendation to cease the trial on efficacy grounds only in the presence of very strong (P<0.001) interim evidence of a difference between groups in the rate of the primary outcome.

In addition, relevant event rates in enrolled infants will be compared with the background rates in data from the ANZNN database for extremely preterm infants, and recommendations for change in sample size made if a substantial disparity is noted.

At each meeting of the DMSC, the ethical position in relation to further randomisation will be considered based on results of any other randomised controlled trials combining intra-tracheal corticosteroid with exogenous surfactant as an early preventative therapy for BPD.

2.22.2 Adverse Events
Safety reporting from the PLUSS Trial will follow standards from the 2016 recommendations of the National Health and Medical Research Council, Australia.54

An ADVERSE EVENT (AE) is any untoward medical occurrence in a patient or clinical trial participant administered a pharmaceutical product and that does not necessarily have a causal relationship with this treatment.

An ADVERSE REACTION (AR) is any untoward and unintended response to an investigational medicinal product related to any dose administered.

A SERIOUS ADVERSE EVENT (SAE)/SERIOUS ADVERSE EVENT (SAR): adverse events/reactions are considered serious if they threaten life or function. Due to the significant information they provide, SAEs require expedited reporting. SAEs/SARs are defined as any adverse event which
• Results in death
• Is life threatening (at the time of the event)
• Requires inpatient hospitalisation or results in prolongation of the existing hospitalisation
• Results in persistent or significant disability

Note: Life-threatening refers to an event in which the participant was at risk of death at the time of the event. It does not refer to an event that hypothetically might have caused death if it were more severe.

Medical and scientific judgement should be exercised in deciding whether an adverse event/reaction should be classified as serious in other situations. Important medical events that are not immediately life-threatening or do not result in death or hospitalisation but may jeopardise the participant or may require intervention to prevent one of the other outcomes listed in this definition should also be considered serious.

A SUSPECTED UNEXPECTED SERIOUS ADVERSE REACTION (SUSAR) is an adverse reaction that is related to the drug that is both serious and unexpected.

2.22.3 Additional safety issues requiring expedited reporting
The following definitions describe additional safety events that require expedited reporting to stakeholders including the Sponsor, Investigators, HRECs/IRBs, local governance offices, the TGA and Competent Authorities/Regulatory Agencies, as per your local country-specific laws and regulations.

A SIGNIFICANT SAFETY ISSUE (SSI) is a safety issue that could adversely affect the safety of participants or materially impact on the continued ethical acceptability or conduct of the trial

An URGENT SAFETY MEASURE (USM) is a measure required to be taken in order to eliminate and immediate hazard to a participant’s health or safety.

2.22.4 Assessment and documentation of adverse events in the PLUSS trial
For the purposes of the PLUSS study, the Site Principal Investigator is responsible for recording all the protocol defined AE/AR during the 14 days after the first intervention. We will record within the CRF the occurrence of the following AEs/ARs, that will be reviewed by the DSMB at their planned safety analyses:

• Hyperglycaemia > 10mmol/L, and/or receiving insulin treatment
• Highest systolic and diastolic blood pressure recorded, and whether the infants received antihypertensive medications
• Newly-diagnosed suspected or proven sepsis treated with intravenous antibiotics/antifungals
• Pneumothorax requiring drainage (needle thoracocentesis and/or intercostal catheter insertion)
• Gastrointestinal haemorrhage defined as fresh blood aspirated from an indwelling gastric tube
• Oral candidiasis
• Clinical diagnosis of pulmonary haemorrhage (eg. blood in aspirate from endotracheal tube)
• Severe IVH (grades III or IV)
For the PLUSS trial, the following events will be reported as SAEs/SUSARs to the DSMB within 7 days, regardless of whether they meet criteria:
- Death
- Spontaneous intestinal perforation
- Serious events that do not result in death:
  - The need for cardiopulmonary resuscitation (chest compressions) and/or administration of adrenaline/epinephrine (for resuscitation) within 24 hours of the intervention
  - Any clinical deterioration of an infant requiring escalation of treatment that the treating clinician considers is secondary to the study intervention

2.22.5 Reporting of SAEs and SUSARs
The Site Principal Investigator is responsible for reporting all SAEs and SUSARs occurring from enrolment to discharge to the Clinical Trial Co-ordinating Centre within 24 hours of the investigator becoming aware of the event using an SAE form.

SAEs must be reported by completing the PLUSS SAE Form and emailing completed and signed forms to the following:

<table>
<thead>
<tr>
<th>Report To:</th>
<th>Email To:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sponsor c/o</td>
<td><a href="mailto:pluss@thewomens.org.au">pluss@thewomens.org.au</a></td>
</tr>
<tr>
<td>PLUSS Clinical</td>
<td></td>
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<tr>
<td>Co-ordinating</td>
<td></td>
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<tr>
<td>Centre</td>
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</tbody>
</table>

The Site Investigator is ultimately responsible for reporting the SAE and must sign the final SAE Report form. Should the Investigator not be available to sign the initial SAE report form within the required 24-hour reporting time period, a comment to this effect must be written on the report and the report signed by the clinician attending to the participant at the time and emailed to the Sponsor. The Investigator must sign the SAE form at the next earliest possible convenience and the SAE report form re-sent to the Sponsor.

SAE Report Form Submission

<table>
<thead>
<tr>
<th>Guidelines Initial Report</th>
<th>Within one working day/24 hours of discovery or notification of the event. If the reporting of an SAE is delayed by more than 24 hours, an explanation must be provided.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Incomplete Reports</td>
<td>If all details are not available at the time of the initial report a completed report must be sent within the next 10 days.</td>
</tr>
<tr>
<td>Updated Report</td>
<td>If the event is not resolved (or ‘on-going’) at the time of the initial report, the SAE Form must be submitted every 30 days until the event is resolved, death has occurred or the condition has stabilised. If a change occurs in a stable condition (i.e. either worsens or improves), then a new SAE Form should be emailed.</td>
</tr>
</tbody>
</table>

The site principal investigator must:
- Determine whether an AE is ‘Serious’ (refer to section 2.19.2.1)
• For SAEs, the Investigator must then ascertain the suspected cause
• The relatedness (attribution) to the SAE must be recorded in the patients’ medical records and reported on the PLUSS SAE form.

All SAEs that have not resolved by the end of the study, or that have not resolved upon discontinuation of the patients participation in the study, must be followed until any of the following occurs:

• The event resolves
• The event stabilises
• The event returns to baseline, if a baseline value/status is available
• The event can be attributed to agents other than the study drug or to factors unrelated to study conduct
• It becomes unlikely that any additional information can be obtained (patient or health care practitioner refusal to provide additional information, lost to follow-up after demonstration of due diligence with follow-up efforts).

2.22.6 Assessing the relatedness (causality) of an SAE
All SAEs must have their relationship to the trial intervention assessed by the investigator who evaluates the adverse event based on temporal relationship and his/her clinical judgment. The degree of certainty about causality will be graded using the categories below. In a clinical trial, the intervention should always be suspected.

The relationship of the event to the trial intervention will be assessed as follows:

• Unrelated: There is no association between the trial intervention and the reported event. SAEs in this category do not have a reasonable temporal relationship to exposure to the intervention, or can be explained by a commonly occurring alternative aetiology.
• Possible: The event could have cause or contributed to the SAE. SAEs in this category follow a reasonable temporal sequence from the time of exposure to the intervention and/or follow a known response pattern to the intervention, but could also have been produced by other factors.
• Probable: The association of the event with the trial intervention seems likely. SAEs in this category follow a reasonable temporal sequence from the time of exposure to the intervention and are consistent with the known action of the intervention, known or previously reported adverse events related the intervention, or judgement based on the investigator’s clinical experience.
• Definite: The SAAE is a consequence of administration of the trial intervention. SAEs in this category cannot be explained by progression of the condition, concurrent illness or medication, or other factors. Such events may be widely documented as having an association with the intervention.

In addition all deaths will be reviewed (blinded to group allocation) by the DSMB soon after they occur. The Trial Coordinator (or nominee) will report all deaths to the DSMB Chair within 7 calendar days of them occurring, when all required information regarding the death is available. The DSMB will review all deaths within 7 working days of them receiving the report. Preterm birth and subsequent neonatal intensive care is associated with many risks including severe morbidity and
mortality. Many of these risks overlap with events that would or could be an SAE/SSI due to the trial interventions. Death is also a primary outcome of the PLUSS Trial. For an independent overview of all deaths, the DSMB will assign the likelihood of the death being related to the study intervention and whether the death was respiratory-related.

SAEs must be reported until final hospital discharge or death. SAE reports should be reported as per the procedure documented in the Study Manual.

2.22.7 Sponsor-Investigator reporting procedures
The Sponsor-Investigator must assess and categorise the PLUSS SAE Report form received from any Site Principal Investigator and report these to all Site Principal Investigators and the approving HRECs in accordance with the NHMRC’s ‘Safety monitoring and reporting in clinical trials involving therapeutic goods’ (November 2016) and any additional requirements of the approving HREC. All safety reports must clarify the impact of the safety event on participant safety, trial conduct and trial documentation.

The Sponsor-Investigator is responsible for the following reporting to all Site Principal Investigators and approving HREC(s):

1. All SSIs that meet the definition of a USM within 72 hours of becoming aware of the issue.
2. All other SSIs within 15 calendar days of instigating or becoming aware of the issue
3. For SSIs leading to an amendment of trial documentation:
   a. Submit details of the SSI without undue delay and no later than 15 calendar days of becoming aware of the issue.
   b. Submit amendment to the HREC without undue delay.
4. For SSIs leading to temporary halt or early termination of a trial for safety reasons:
   a. Communicate reasons, scope of halt, measures taken, further actions planned without undue delay and no later than 15 calendar days of decision to halt.
   b. For a temporary halt, notify the Site Principal Investigators and approving HRECs when the trial restarts, including evidence that it is safe to do so.

The Sponsor is responsible for providing an annual safety report, including a summary of the evolving safety profile of the trial.

2.23 Safety and pharmacokinetics of intra-tracheal budesonide administered with surfactant
Budesonide has high first-pass metabolism such that any drug that refluxes into the pharynx and is swallowed will have very limited absorption from the gastrointestinal tract. Thus, systemic absorption occurs primarily from the respiratory tract. Budesonide is a relatively hydrophilic corticosteroid and is absorbed into the systemic circulation within minutes of deposition in the lung. However, budesonide undergoes extensive (70% to 80%) reversible conjugation with fatty acids (e.g., budesonide pulmitate) leading to intracellular accumulation in epithelial cells, effectively forming a local depot of corticosteroid in the lung. Budesonide conjugates are gradually hydrolysed and free budesonide is generated. This form of airway selectivity is specific to budesonide and is not seen with other synthetic corticosteroids such as fluticasone, which lack a 21-hydroxyl group. Further, unlike beclomethasone, budesonide is not transformed to inactive metabolites within the lung. In vitro and in vivo studies have shown that using surfactant as a vehicle dramatically
improves the distribution of corticosteroids in the distal lung, and this combined with the selective pharmacokinetic properties of budesonide make budesonide and surfactant an ideal candidate anti-inflammatory treatment in the neonatal lung.

Once in the systemic circulation, budesonide is extensively metabolised in the liver by cytochrome p450 3A enzymes to inactive compounds (predominantly 16α-hydroxy prednisolone). Although preterm infants have lower activity of these enzymes than adults, they appear to readily clear budesonide from the circulation. Studies in preterm infants and lambs using surfactant as a vehicle for budesonide show that systemic uptake of budesonide peaks 30 to 60 min after installation but plasma concentrations remain very low (Cmax 20-30 ng/ml). The half-life is between 4.1 to 4.7 hours, which is considerably shorter than for other neonatal drugs, again suggesting rapid clearance. Budesonide is detectable in lung tissue for up to 24 hours after a single dose and anti-inflammatory effects last for at least 72 hours, indicating considerable airway selectivity for this drug. Importantly, in preterm lambs, budesonide was not detected in either white or grey matter in the brain, making central nervous system effects unlikely. One study in preterm infants suggested that mean cortisol concentrations were slightly lower at 8 hours in those exposed to intra-tracheal budesonide but this difference was not statistically significant (8.3 vs 12.6 ng/ml).

2.23.1 Drug monitoring
A parallel sub-study (separate protocol) will be designed and conducted at the two main recruiting centres, Melbourne and Auckland, to monitor systemic absorption of budesonide, and any acute effect on endogenous cortisol production. This sub-study does not form part of the current trial protocol and will be submitted separately for ethical approval.

2.24 Funding
The PLUSS Trial has been funded by an NHMRC. Project Grant (No. 1158555) for 5 years commencing January 2019. Chiesi Farmaceutici, Parma, Italy has agreed to reimburse the cost of surfactant (Curosurf) used in the trial to centres through an unrestricted grant for the trial.

3 SIGNIFICANCE
BPD is the most important pulmonary complication in extremely preterm infants, occurring in about 50% of survivors to 36 weeks PMA, with few interventions shown to safely reduce it. BPD is associated with early death and long-term adverse pulmonary health and neurodevelopmental outcomes in survivors.

We propose a highly-powered, pragmatic and inclusive trial of intra-tracheal corticosteroid (budesonide), using the most commonly-used surfactant (Curosurf) as a vehicle to deliver it to the lungs.

If this cheap, easy intervention is effective and safe, it will be rapidly accepted into clinical practice around the world, impacting the care of tens of thousands of these vulnerable infants.
4 References