PROTOCOL

BLUEPRINT

BLUEPRINT: Predicting Long-term Outcomes of Prematurity from Early Life Events

Protocol Number: 107608

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Document history:

Version Number and Date	Summary of changes
V1.0 24 April 2024	Original Version
V1.1 30 April 2024	Revised version following Research Education and Governance review (Royal Children's Hospital Human Research and Ethics Committee).
V1.2 30 May 2024	Revised version following HREC committee review (Royal Children's Hospital Human Research and Ethics Committee).

CONFIDENTIAL

Statement of Compliance

This study will be conducted in compliance with all stipulation of this protocol, the conditions of the ethics committee approval, the NHMRC National Statement on Ethical Conduct in Human Research (2007 and all updates), applicable national and local regulations and in the spirit of the Integrated Addendum to ICH E6 (R1): Guideline for Good Clinical Practice E6 (R2), dated 9 November 2016 annotated with TGA comments.

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PROTOCOL SYNOPSIS

TITLE	Defining Early Life Respiratory Phenotypes to Predict Long-term outcomes of Prematurity: An observational study (the BLUEPRINT Study)
STUDY DESCRIPTION	A prospective longitudinal observational cohort study using clinical, lung imaging and plasma protein data to define the early postnatal respiratory endotype following preterm birth, and to determine the relationship with bronchopulmonary dysplasia (BPD) and 1- and 2-year respiratory outcomes.
OBJECTIVES	The primary objective of the BLUEPRINT Study is to comprehensively define the respiratory phenotype and endotypes using observations from the first 7 days after birth in preterm infants born between 22+0 and 31+6 weeks' gestation. The secondary objectives of this study are: 1. To compare the respiratory endotypes associated with different respiratory outcomes using observational data at 72 hours after birth in preterm infants born between 22+0 and 31+6 weeks' gestation. 2. To develop the first functional classification of preterm respiratory disease using a combination of clinical, lung imaging and molecular biomarkers. 3. Determine how specific early preterm endotypes relate to a diagnosis of BPD to develop a predictive modelling tool for the early diagnosis of BPD that can be used in everyday clinical practice. 4. Determine how specific early preterm endotypes relate to outcomes of the primary NICU admission, including duration of invasive mechanical ventilation and supplementary oxygen support, mortality, need for supplementary oxygen at discharge home. 5. To characterise the respiratory health of preterm infants in the first 2 years (corrected) after birth. 6. To characterise overall childhood health of preterm infants in the first 2 years (corrected) after birth. 7. To determine the relationship between initial respiratory endotype, BPD status and early childhood respiratory health at 1 and 2 years of corrected age. 8. To determine the performance of early life endotypes against a 36-week diagnosis of BPD in predicting respiratory outcomes at 1 and 2 years of corrected age. 9. To determine the relationship between LUS-derived measures of respiratory status at 14 and 28 days after birth and BPD status. 10. To determine the relationship between EIT-derived measures of respiratory status at 14 and 28 days after birth and BPD status.

	11. To use plasma proteomics to identify proteins whose expression is altered in the first 7 days of life in infants who later are diagnosed with BPD. 12. To use plasma proteomics to identify expression pathways associated with specific preterm respiratory endotypes in samples obtained at 72 hours and 7 days post-birth. 13. To determine the impact of clinical interventions on the development of the respiratory phenotype.
OUTCOMES AND OUTCOME MEASURES	The primary outcome is identification of functional endotypes defined from clinical, plasma protein and lung imaging measures using cross-sectional and longitudinal latent class analysis. The secondary outcomes include the functional endotype at 72 hours, the relationship between early life phenotypes, proteomic and lung imaging and later BPD status and respiratory health at 1 and 2 years of corrected age, and the development of prediction models for BPD using the early life respiratory endotypes.
EXPOSURES	Eligible and enrolled infants will have clinical data relating to their antenatal, maternal and postnatal respiratory and neonatal intensive care course collected at pre-defined time points between birth and NICU discharge. In addition, a maximum of 0.5 ml of blood will be collected at 72 hours and 7 days, and a brief LUS and EIT image of the lung performed at 48 and 72 hours, 7, 14 and 28 days, and then at 36 weeks' postmenstural age (BPD diagnosis). Lung imaging and assessment of respiratory health using a standardised questionnaire will be repeated at 1 and 2 years corrected age in preterm survivors.
POTENTIAL CONFOUNDING FACTORS	Maternal and antenatal factors and management, clinical decisions regarding respiratory support (site and clinician differences in guidelines and management), preterm non-respiratory complications (such as infection, neurological disease, necrotising enterocolitis, jaundice).
STUDY POPULATION	Infants born between 22+0 and 31+6 weeks' gestation admitted to a participating centre from whom prospective parental informed consent can be obtained will be considered eligible. If parental consent cannot be obtained or if lung imaging or blood sampling would compromise clinical care or well-being, the infant will be considered ineligible for participation in this study. A sample size of 550 infants will allow for prediction models of BPD using up to 12 variables from the respiratory phenotype data collected in the first 7 days after birth with a C-index of 0.75 (assuming 5% lost to follow up). This sample size will have sufficient power to address the endotyping and respiratory health

	objectives of the study at 1- and 2-years corrected age, with account for anticipated loss to follow up rates.
DESCRIPTION OF SITES ENROLLING PARTICIPANTS	All Level 6 NICUs in Melbourne, Victoria (Australia) will be invited to participate (5 sites maximum).
RECRUITMENT PERIOD	Approximately 5 years after first infant enrolled.
FOLLOW-UP PERIOD	2 years, assessments at 1- and 2-years (corrected).
PARTICIPANT DURATION	2 years, corrected age from birth.

GLOSSARY OF ABBREVIATIONS

ABBREVIATION	***TERM***
AE	Adverse Event
ANOVA	Analysis of Variance
ANZNN	Australian and New Zealand Neonatal Network: a collaborative network which monitors and reports on the care of high-risk newborns in all Australian and New Zealand neonatal intensive care units.
BPD	Bronchopulmonary dysplasia: a form of chronic lung disease that affects preterm infants
CLD	Chronic Lung Disease
CCe	Coordinating Centre
CRF / eCRF	Case Report Form / electronic Case Report Form
CXR	Chest Radiography
DCCe	Data Coordinating Centre
DMS	Data Management System
DSMC	Data Safety Monitoring Committee
EDTA	Ethylenediaminetetraacetic acid: used as an anticoagulant in blood tubes
EIT	Electrical Impedance Tomography
EMR	Electronic Medical Record
ETT	Endotracheal Tube
FiO_2	Fraction of Inspired Oxygen
GA	Gestational age
GCP	Good Clinical Practice
HR	Heart Rate
HREC	Human Research Ethics Committee
IVH	Intraventricular Haemorrhage
LCA	Latent Class Analysis
LUS	Lung Ultrasound
LUSS	Lung Ultrasound Score

MCRI	Murdoch Children's Research Institute
MedDRA	Medical Dictionary for Regulatory Activities
NHMRC	National Health and Medical Research Council
NICU	Neonatal Intensive Care Unit
NIV	Non-invasive Ventilation
ORT	Oxygen Reduction Test
PDA	Patent Ductus Arteriosus
PI / CPI	Principal Investigator / Coordinating Principal Investigator
PICF	Participant (parent) Information and Consent Form
PMA	Post-menstrual age
QA	Quality Assurance
QC	Quality Control
REDCap	Research Electronic Data capture: a secure web application for building and managing online databases
RGO	Research Governance Office
RCH	Royal Children's Hospital (Melbourne)
RWH	Royal Women's Hospital (Melbourne)
SAP	Statistical Analysis Plan
SSC	Study Steering Committee
SEC	Study Executive Committee
SoA	Schedule of Assessments
SOP	Standard Operating Procedure
SpO_2	Peripheral Oxygen Saturation

INVESTIGATOR AGREEMENT & SIGNATURE PAGE

I have read the protocol entitled "Defining Early Life Respiratory Phenotypes to Predict Long-term outcomes of Prematurity: An observational study (the BLUEPRINT Study)".

By signing this protocol, I agree to conduct the study, after approval by a Human Research Ethics Committee or Institutional Review Board (as appropriate), in accordance with the protocol and:

- the principles of the Declaration of Helsinki
- the NHMRC National Statement on Ethical Conduct in Human Research (2007 and all updates)
- the Australian Code for the Responsible Conduct of Research (NHMRC, 2007 and all updates)
- and in the spirit of the good clinical practice guidelines adopted by the TGA [Integrated Addendum to ICH E6 (R1): Guideline for Good Clinical Practice E6 (R2), dated 9 November 2016 annotated with TGA comments].

Changes to the protocol will only be implemented after written approval is received from the Human Research Ethics Committee or Institutional Review Board (as appropriate), with the exception of medical emergencies.

I will ensure that study staff fully understand and follow the protocol and evidence of their training is documented on the study training log. I agree to ensure that the confidential information contained in this document will not be used for any other purpose other than the evaluation or conduct of the clinical investigation without the prior written consent of the Sponsor.

…… ……/……/…….
Signature *Date (DD/MM/YY)*

NAME:	
ROLE:	
INSTITUTION:	

…… ……/……/…….
Signature *Date (DD/MM/YY)*

NAME:	Professor David Tingay
ROLE:	Coordinating Principal Investigator
INSTITUTION:	Royal Children's Hospital, Murdoch Children's Research Institute

1 ADMINISTRATIVE INFORMATION

1.1 Registration of observational research

This trial has been submitted for registration on ANZCTR (ACTRN Request Number 382577; submitted 27 April 2024). Universal Trial Number U1111-1268-7555.

1.2 Sponsor

On behalf of the Sponsor (MCRI), the Coordinating Principal Investigator (Coordinating PI) (Professor David Tingay) will undertake and/or oversee those Sponsor responsibilities delegated by the Sponsor.

The Coordinating PI will also ensure that each investigator at the participating sites conducts the study in compliance with the protocol, relevant approvals and regulatory requirements.

Study Sponsor	Murdoch Children's Research Institute (MCRI)
Contact name	Professor David Tingay
Address	50 Flemington Road, Parkville, Victoria 3052

1.3 Expected duration of study

It is expected that 550 infants from 5 Victorian Tertiary level NICUs across Victoria will be recruited over a 3-year period from the date of when the first infant is enrolled into the study. Study infants will remain on-study from date of enrolment until 2 years corrected age. The study is expected to be completed when the last study child has completed a 2-year assessment (±3-month window for assessment scheduling).

1.4 Contributorship

Name	**Summary of contribution**
Prof David Tingay	Study PI, wrote first draft of protocol and revisions
Prof Peter Davis	Study CI, contributed to all drafts
Dr Prue Pereira-Fantini	Study CI, lead for proteomics sections, contributed to all drafts
Dr Arun Sett	Study CI, lead for lung imaging sections contributed to all drafts
Dr Merrin Pang	Study Coordinator, contributed to all drafts and revisions
Prof Jeanie Cheong	Study CI, contributed to all drafts
Dr Shivanth Shanthikumar	Study CI, contributed to all drafts
Dr Kate Hodgson	Study CI, contributed to all drafts

1.5 Source of funding

This study is funded by a NHMRC Clinical Trials and Cohorts Scheme Grant (GNT2024039).

1.6 Stakeholder involvement

Consumer input was sought in the study development from Marijn Mees and Joris Steeman from the ByBoet Foundation, who are also listed as Associate Investigators on the original BLUEPRINT NHMRC grant application. Both will have ongoing involvement in study conduct and knowledge dissemination and be members of the Study Steering Committee. Consumers' top priorities for research regularly include improving respiratory outcomes for preterm infants, including in the recent James Lind Alliance Priority Setting Partnership Project for the most preterm infants (MCRI co-lead).

1.7 Study Locations

SPONSOR SITE			
Site	**Address**	**Contact Person**	**Email**
Murdoch Children's Research Institute (MCRI)	50 Flemington Road, Parkville, VIC 3052, Australia	Dr Merrin Pang Study coordinator	merrin.pang@mrci.edu.au
PARTICIPATING INSTITUTIONS			
Site	**Address**	**Principal Investigator**	**Email**
The Royal Children's Hospital (RCH)	50 Flemington Road, Parkville, VIC 3052, Australia	Professor David Tingay	david.tingay@rch.org.au
The Royal Women's Hospital (RWH)	20 Flemington Road, Parkville, VIC 3052, Australia	Dr Kate Hodgson	kate.hodgson@thewomens.org.au
Monash Children's Hospital	246 Clayton Rd, Clayton, VIC 3168, Australia	Professor Rodney Hunt	rod.hunt@monash.edu
Joan Kirner Women's & Children's, Sunshine Hospital	176 Furlong Rd, St Albans, VIC 3021, Australia	Dr Arun Sett	arun.sett@wh.org.au
Mercy Hospital for Women	163 Studley Rd, Heidelberg, VIC 3084, Australia	Dr Shiraz Badurdeen	shiraz.badurdeen@mcri.edu.au

COLLABORATING INSTITUTIONS			
Baker Heart and Diabetes Institute	75 Commercial Rd, Melbourne, 3004 VIC, Australia	Associate Professor David Greening	david.greening@baker.edu.au

2 INTRODUCTION AND BACKGROUND

2.1 Background and rationale

Globally, 15 million babies are born preterm each year, and 1 million die.(1) Respiratory disease is the greatest cause of illness for these infants;(2) almost 75% born before 32 weeks' gestation receive assisted respiratory support. However, surviving to discharge home is not the endpoint for this respiratory burden. Ventilation of the underdeveloped lung is a double-edged sword; necessary for survival but risk long-term injury to the lungs.(2) Despite 40 years of advances in respiratory support, many preterm infants develop early lung injury that eventually leads to chronic lung disease (CLD) throughout infancy, childhood and in adult life. This burden is increasing.(3, 4)

The accepted measure of in-hospital respiratory morbidity, bronchopulmonary dysplasia (BPD) diagnosed at 36 weeks' postmenstrual age (PMA)(5), does not reliably predict respiratory complications in later life and has often been conflated to represent CLD status.(6) Irrespective of BPD status, childhood respiratory function in preterm survivors is now worse than 25 years ago. Abnormal childhood respiratory function is associated with adult obstructive CLD and deteriorating lung function.(7, 8) Necessitating urgent re-evaluation of how early preterm respiratory disease is defined, identified and managed, in order to reduce the ensuing burden on infants, families and health services.

Avoiding the trajectory towards CLD in later life requires modulation of the early life events that initiate the lung injury/inflammation cascade at and shortly after birth (**Figure 2a**). Currently, these events are defined from crude measures that poorly categorise the complexity of prematurity and NICU management. To provide the right lung protective treatment, at the right time, clinicians need precise guidance to make decisions that avoid or modify the initial disease process.

This study aims to bring together the tertiary-level NICUs in Victoria to:

1. Define early-life respiratory phenotypes in preterm infants,
2. Link these phenotypes to medium (in-hospital) and long-term respiratory outcomes, specifically BPD and respiratory health at 1 and 2 years of corrected age, and
3. Integrate protein biomarkers, lung imaging and clinical tools to define the continuum of modifiable mechanical-molecular-clinical interactions occurring in early preterm life.

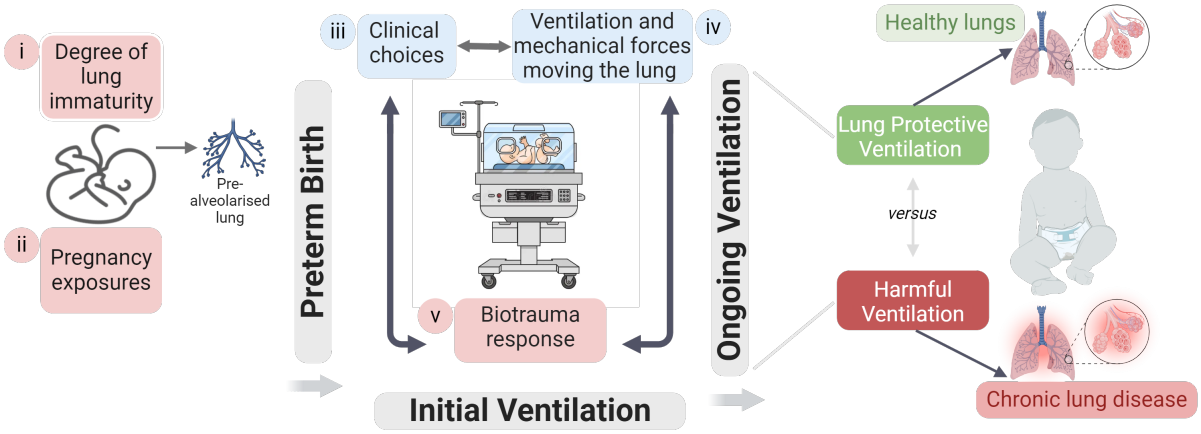

Figure 2a Preterm respiratory health is determined by a cascade of differing early life experiences that alter the trajectory of lung growth during childhood and risk of chronic lung disease in later life. Developing meaningful long-term benefits requires targeted disease modifying therapies using a multidimensional approach.

The specific purpose of this prospective observational cohort study is to:

1) Develop the first functional classification of preterm respiratory disease: Comprehensively define the preterm respiratory phenotype in the first 7 days of life using a range of biomarkers, including circulating proteins, imaging, and clinical information, and delineating the influence of gestation, maternal factors, and clinical interventions on marker expression.

2) Accurately predict BPD: Determine how specific early preterm phenotypes relate to a diagnosis of BPD and develop a predictive modelling tool for the early diagnosis of BPD that can be implemented in daily clinical practice. By doing so, we hope to delineate the first set of practical repeatable measures that clinicians can use to predict the trajectory towards BPD, compare therapeutic benefits and potentially inform decisions that will improve NICU care.

3) Improve our understanding of the longer-term implications of early respiratory disease: Characterise the respiratory health of preterm infants in the first 2 years after birth. By doing so, defining the relationship between initial respiratory phenotype, BPD status and early childhood respiratory health. Providing a robust approach to continually assess infant lung function and lung growth from the NICU into childhood that may offer better utility for determining long-term respiratory risk from prematurity and provide parents with relevant expectations regarding their child's health.

Together, our study aims will address the key knowledge gaps identified in the '*American Thoracic Society Statement on the Care of the Child with Chronic Lung Disease of Infancy and Childhood*':

- What are the best methods to describe BPD?
- What is the natural history of lung function changes in chronic lung disease of infancy?
- What is the relative importance of early injury events on chronic lung disease in childhood?

Achieving these aims will represent a major and enduring conceptual shift in the approach to neonatal lung protection relevant to all modern NICU care worldwide.

Defining the early origins of BPD progression is essential to improving outcomes

Reducing the rate of BPD continues to challenge neonatologists, with few therapies demonstrating benefit to date.(2, 9) BPD results from injury initiated in the early neonatal period and is exacerbated by other morbidities such as sepsis. Currently, BPD is diagnosed at 36 weeks' PMA,(2, 10-12) well after the initiating events. This late diagnosis limits the application of interventions designed to alter respiratory outcomes.(9) Several clinical factors (e.g. gestation, sex, chorioamnionitis, duration of mechanical ventilation and supplemental oxygen needs) are associated with BPD.(2) However, none alone or in combination have shown to accurately predict the risk.(9, 11) The reasons for this inaccuracy may lie in the use of measures that are too crude to allow prediction of this complex disease. This is perpetuated by a 'one-size-fits-all' approach to applying various respiratory therapies available in the NICU (e.g. continuous positive applied pressure, high-flow nasal cannula, intubation).(2) Therefore, to progress and reliably predict respiratory risks, there is a pressing need to identify these complex early phenotypes at a biological systems level. Such information will not only serve as targets for interventions aimed at ameliorating lung injury, but to also guide respiratory decisions in the crucial period shortly after birth when strategies to prevent BPD may have the most benefit.

BPD is the result of a multi-modal inflammatory cascade of events but is defined purely on clinical status. Our pilot work suggests that the inclusion of both protein and lung function (imaging) measures (with clinical data) in the first 7 days can differentiate respiratory phenotypes that improve the accuracy of BPD prediction. This is the critical first step in implementing stratified lung protective respiratory care based on evidence before the initial injury-inflammation cascade becomes entrenched.

Defining the landscape of early preterm life: many phenotypes, many choices, many outcomes

Survival following birth before 32 weeks' gestation requires the lung to ventilate whilst in a saccular or canalicular stage of development, before the formation of alveoli and production of surfactant. Broadly, preterm lung injury is due to multiple injurious stimuli applied to the structurally immature and surfactant deficient lung in this early postnatal period. Maternal (e.g. chorioamnionitis), infant (e.g. weight, sex, gestation) and immune factors alter the likelihood of lung injury.(2, 13) Hence, the development of preterm lung injury is a multi-factorial process; a complex interplay between numerous pathophysiological and patient-derived factors. The injury process continues to evolve, influenced by treatment choices in the NICU (e.g. intubation, surfactant therapy). However, as we have shown, the pathological process (biotrauma) is unique to each infant but can also be defined using a combination of molecular (proteomics), imaging and clinical data.

Our Neonatal Proteomics program has applied advanced mass spectrometry to identify the mediators, pathways and mechanisms that define the biotrauma response that occurs within the preterm lung.(14-16) Using our bioinformatics approach,(14, 16) we have determined the molecular events associated with specific respiratory strategies at different gestations.(14, 17, 18) Importantly, because our quantitative proteome approach is comprehensive (analysis of high and low abundant protein species) and unbiased (untargeted), we have identified new regulators and pathways related to specific clinical interactions resulting in injury (and vice versa) not detected using traditional methods.(16)

Measuring lung function at a resolution that links injurious lung states (for example atelectasis and volutrauma) with clinical treatments has previously been impossible in infants. We have utilised two high-resolution bedside imaging techniques in the NICU; lung ultrasound (LUS) and electrical impedance tomography (EIT).(19-21) Both provide radiation-free, repeatable, detailed breath-by-breath imaging of aeration. EIT also images regional lung ventilation and mechanics. We have shown that LUS and EIT assessments of ventilation in early preterm life predicts BPD risk,(20) and can direct clinical respiratory decision making in the NICU.(22, 23) We have also shown that standard EIT measures

correlate with the molecular and proteome changes occurring within the preterm lung associated with injurious and protective respiratory therapies.(18, 23) Importantly, both EIT and LUS can be repeated through childhood.

Complex disease trajectories require comprehensive mapping techniques to characterise them

Addressing the complexity of early lung injury requires a sophisticated, multi-modal mapping approach. Biomarker data are captured at the cellular level (mass spectrometry of plasma) and system level (EIT and LUS) and then integrated with clinical information. We will use bioinformatics techniques to map these interactions. By doing so, we hope the methods we develop in this study may 1) improve our understanding of the best timing for interventions, 2) identify new therapeutic options and tools for defining an intervention's success, failure and/or suitability, and 3) increase our understanding of the impact of secondary perinatal and maternal factors on preterm lung injury. A similar approach has been successfully employed in paediatric and adult acute respiratory distress syndrome (a functionally similar condition to preterm acute lung disease).(24, 25)

Is it time to re-think our reliance on BPD to define preterm respiratory outcomes?

The accepted definition of BPD has changed as neonatal medicine has evolved. Originally a pathological definition, the current definition is based upon a level of respiratory support and oxygen dependency at 36 weeks' PMA.(2, 5, 11, 12) No definition is ideal, and all are based upon a single trait (e.g. respiratory support) that narrows categorisation and ignores the multi-modal impact of preterm birth on the lung.(11, 12, 26) Most BPD definitions were developed in an era in which babies born at 22- and 23-weeks' gestation rarely survived. As BPD remains the primary outcome measure for large neonatal trials, this fundamental problem has arguably limited the development of new respiratory treatments. More concerningly, childhood respiratory function does not always relate to BPD diagnosis.(3, 4) Poor compliance with traditional pulmonary function tests in early childhood precludes reliable assessment of respiratory function. This creates a period of clinical and parental uncertainty during the early years of childhood. Our study standardises lung function assessments before and after NICU care to better understand how biological and physiological events in the early newborn period shape the trajectory of lung growth and health outcomes in later life. This may aid in understanding important respiratory factors not currently recognised using current BPD diagnostic criteria, and provide the missing link between BPD, as currently defined, and impaired respiratory function in childhood.

Summary of BLUEPRINT Study Design

The BLUEPRINT study is a longitudinal prospective observational study aiming to recruit preterm infants from a maximum of 5 tertiary NICUs in Victoria (Australia) and follow them from the early NICU experience (24 hours to Day 14), through to 36 weeks' PMA or discharge and then an annual review at 1 and 2 years corrected age to assess respiratory health.

Detailed clinical data on the antenatal, delivery and initial NICU course (24-, 48-, 72-hours, 7 and 14 days) will be collected, focusing on respiratory care and complications of prematurity. BPD status will be collected at 36 weeks' PMA, as well as data on known complications of preterm birth and NICU management at discharge.

Blood samples will be collected at approximately 72 hours and Day 7 for mass spectrometry analysis of the plasma proteome.

LUS and EIT imaging will be performed at 24, 72 hours and Day 7, and then repeated at Days 14 and 28, and 36-weeks' PMA.

Families who agree to follow-up after hospital discharge will be asked to attend the MCRI research clinic for assessment of respiratory health (clinical assessment and standardised questionnaire) at 1 and 2 years corrected age. LUS and EIT imaging will be repeated at these appointments.

2.2 Definitions

The following definitions are applicable to this study:

Phenotype = any observable characteristic or trait of a disease, such as the clinical presentation of an individual. There is no implication of a mechanism.

Endotype = an endotype is defined by a distinct function or pathobiological mechanisms that manifest in or are exhibited by a phenotype of cluster of phenotypes.

An early life preterm respiratory-specific endotype may present with phenotypic clusters of disease. Preterm respiratory-specific endotypes can be specific to a particular time-point and can also be defined longitudinally over the first 2 years after birth (corrected for gestation).

2.3 Study aim(s)

The aim of this study is to describe the respiratory phenotypes and endotypes of infants born preterm in early-postnatal life, who are managed in the NICU using standard respiratory support practices.

By doing so, we aim to:

1) Define the clinical, lung function and molecular characteristics of early respiratory disease in preterm infants.
2) Determine the relationship between these endotype characteristics and later BPD status.
3) Determine the relationship between these endotypes and respiratory health at 1 and 2 years corrected age.
4) Develop predictive modelling tools for the early diagnosis of BPD and respiratory health in the first 2 years after birth.

3 STUDY OBJECTIVES AND OUTCOMES

3.1 Objectives

3.1.1 Primary objective

The primary objective of the BLUEPRINT study is to comprehensively define the respiratory phenotype and endotypes using observations from the first 7 days after birth in preterm infants born between 22+0 and 31+6 weeks' gestation.

3.1.2 Secondary objectives

The secondary objectives of this study are:

1. To compare the respiratory endotypes associated with different respiratory outcomes using observational data at 72 hours after birth in preterm infants born between 22+0 and 31+6 weeks' gestation.

2. To develop the first functional classification of preterm respiratory disease using a combination of clinical, lung imaging and molecular biomarkers.

3. Determine how specific early preterm endotypes relate to a diagnosis of BPD to develop a predictive modelling tool for the early diagnosis of BPD that can be used in everyday clinical practice.

4. Determine how specific early preterm endotypes relate to outcomes of the primary NICU admission, including duration of invasive mechanical ventilation and supplementary oxygen support, mortality, need for supplementary oxygen at discharge home.

5. To characterise the respiratory health of preterm infants in the first 2 years (corrected) after birth.

6. To characterise overall childhood health of preterm infants in the first 2 years (corrected) after birth.

7. To determine the relationship between initial respiratory endotype, BPD status and early childhood respiratory health at 1 and 2 years of corrected age.

8. To determine the performance of early life endotypes against a 36-week diagnosis of BPD in predicting respiratory outcomes at 1 and 2 years of corrected age.

9. To determine the relationship between LUS-derived measures of respiratory status at 14 and 28 days after birth and BPD status.

10. To determine the relationship between EIT-derived measures of respiratory status at 14 and 28 days after birth and BPD status.

11. To use plasma proteomics to identify proteins whose expression is altered in the first 7 days of life in infants who later are diagnosed with BPD.

12. To use plasma proteomics to identify expression pathways associated with specific preterm respiratory endotypes in samples obtained at 72 hours and 7 days post-birth.

13. To determine the impact of clinical interventions on the development of the respiratory phenotype.

3.1.3 Exploratory objectives

The BLUEPRINT study aims to explore the role of precision medicine and prediction modelling in neonatal critical care, and the development of new approaches to understanding long-term respiratory impacts of preterm birth that may address some of the limitations of the use of BPD and the lack of sound methods of defining respiratory health in infancy.

There are some important exploratory objectives that may arise from this study. Specifically:

1. Whether respiratory health and complications of preterm birth can be better defined using an alternative set of diagnostic criteria for BPD that better reflect functional respiratory health and clinical events in the NICU, or a new entity entirely based upon new biomarkers.

2. Whether plasma protein biomarkers can improve the ability to define respiratory trajectory following preterm birth, specifically with relation to response to respiratory care and early identification of complications of preterm birth.

3. Whether LUS and EIT biomarkers can improve the ability to define respiratory trajectory following preterm birth, specifically with relation to type of respiratory care and complications of NICU and preterm birth.

4. Whether heterogeneity of ventilation is a predictor of later BPD and respiratory health at 1 and 2 years (corrected).

5. Whether a series of measures or definition of respiratory health at 1 and 2 years can be developed that would provide a more meaningful representation of the respiratory impact of preterm birth than the diagnosis of BPD.

6. Whether the inclusion of unsupervised plasma proteomics and LUS and EIT may identify novel treatments via identification of previously unexplored concepts and/or molecules that influence preterm respiratory outcomes.

7. Whether the comprehensive clinical, imaging and protein datasets generated may allow for a better understanding of the extrapulmonary impact of preterm respiratory disease.

8. To determine the relationship between maternal and fetal characteristics and plasma protein abundance at 72 hours and 7 days post-birth.

3.2 Outcomes

Table 3a defines the outcomes measures in relation to objectives and confounders.

Table 3a Listing objectives, exposure, outcomes and outcome measures, and confounders

PRIMARY OBJECTIVE
To comprehensively define the respiratory phenotype in the first 7 days after birth of preterm infants born between 22+0 and 31+6 weeks' gestation.
Exposure: Day 7 after birth.
Outcome & outcome measure: Functional endotypes defined from clinical, plasma protein and lung imaging measures using latent class analysis followed by external validation via group-based trajectory modelling.
Confounding factors: Maternal and antenatal factors and management, clinical decisions regarding respiratory support (site and clinician differences in guidelines and management), preterm non-respiratory complications (such as infection, neurological disease, necrotising enterocolitis).
SECONDARY OBJECTIVES
To compare the respiratory endotypes associated with different respiratory outcomes using observational data at 72 hours after birth in preterm infants born between 22+0 and 31+6 weeks' gestation.
Outcome & outcome measure Functional endotypes defined from clinical, plasma protein and lung imaging measures using latent class analysis (LCA) followed by external validation via group-based trajectory modelling.
To develop the first functional classification of preterm respiratory disease using a combination of clinical, lung imaging and molecular biomarkers.
Outcome & outcome measure Temporally specific respiratory phenotypes derived from longitudinal latent class analysis of clinical, lung imaging and molecular biomarker data from all time points of the study.
To determine how specific early preterm phenotypes relate to a diagnosis of BPD and use this delineation to develop a predictive modelling tool for the early diagnosis of BPD.
Outcome & outcome measure

BPD status at 36 weeks' corrected PMA using Walsh Definition and Jensen Grade of Severity and functional endotypes determined above.
SECONDARY OBJECTIVES cont.
To determine how specific early preterm endotypes relate to outcomes of the primary NICU admission, including duration of invasive mechanical ventilation and supplementary oxygen support, mortality, need for supplementary oxygen at discharge home.
Outcome & outcome measure Duration of invasive mechanical ventilation and supplementary oxygen support, mortality and need for supplementary oxygen at discharge home as independent outcomes and functional endotypes determined above.
To characterise the respiratory health of preterm infants in the first 2 years (corrected) after birth.
Outcome & outcome measure Respiratory health status questionnaire, gravity-dependent and right-left lung centre of ventilation and homogeneity index (EIT*) and LUS* score (LUSS) at 1 and 2 years corrected age.
To characterise overall childhood health of preterm infants in the first 2 years (corrected) after birth.
Outcome & outcome measure General health status defined by growth parameters and medical history since discharge and development (PARCA-R) questionnaires.
To determine the relationship between initial respiratory phenotype, BPD status and early childhood respiratory health.
Outcome & outcome measure Respiratory phenotype at 72 hours and Day 7, BPD status and severity grade and Respiratory health outcomes at 1 and 2 years corrected age.
To determine the performance of early life endotypes against a 36-week diagnosis of BPD in predicting respiratory outcomes at 1 and 2 years of corrected age.
Outcome & outcome measure BPD status at 36 weeks' corrected PMA using Walsh Definition and Jensen Grade of Severity, respiratory health at 1 and 2 years corrected age and functional endotypes determined above.
To determine the relationship between LUSS-derived measures* of respiratory status at 14 and 28 days after birth and BPD status.
Outcome & outcome measure

The accuracy of the blindly calculated LUS score proposed by Brat et al(27) in predicting a final diagnosis of BPD at 36 weeks (Yes/No). This score will be used as the primary lung ultrasound score incorporated into the predictive model.
To determine the relationship between EIT-derived measures of respiratory status at 14 and 28 days after birth and BPD status.
Outcome & outcome measure Gravity-dependent and right-left lung centre of ventilation and homogeneity index (EIT*) at 14 and 28 days after birth.
To use plasma proteomics to identify proteins whose expression is altered in the first 7 days of life in infants who later are diagnosed with BPD.
Outcome & outcome measure Difference in plasma protein abundance at 72 hours and 7 days post-birth between infants with and without BPD.
To use plasma proteomics of samples obtained at 72 hours and 7 days post-birth to identify pathways whose expression is associated with specific preterm respiratory endotypes.
Outcome & outcome measure Bioinformatic analysis to determine increased association of endotype-associated proteins with specific pathways and biological processes.
To determine the impact of clinical interventions on the development of the respiratory phenotype.
Outcome & outcome measure Sub-analysis will be performed to identify the impact of clinical interventions on plasma proteome composition using clustering methods, including hierarchal clustering, principal component analysis and k-means clustering and one-way analysis of variance (ANOVA). Clinical interventions will include (but not limited to) surfactant administration, mode of support, ventilator settings, corticosteroid administration, blood transfusion and duration of invasive ventilation.

*Measures to define ventilation and aeration patterns using EIT and LUS are based upon existing guidelines.(23) Both technologies are likely to generate new outcome measures and scoring systems for global and regional ventilation, aeration and lung mechanics during the study period. These outcome measures may be added if 1) they can be extracted from raw imaging data acquired at Scheduled Assessments and 2) they have been considered valid measures in international standards.

Outcome measures are further detailed in Section 6.

4 STUDY DESIGN

4.1 Study design schema

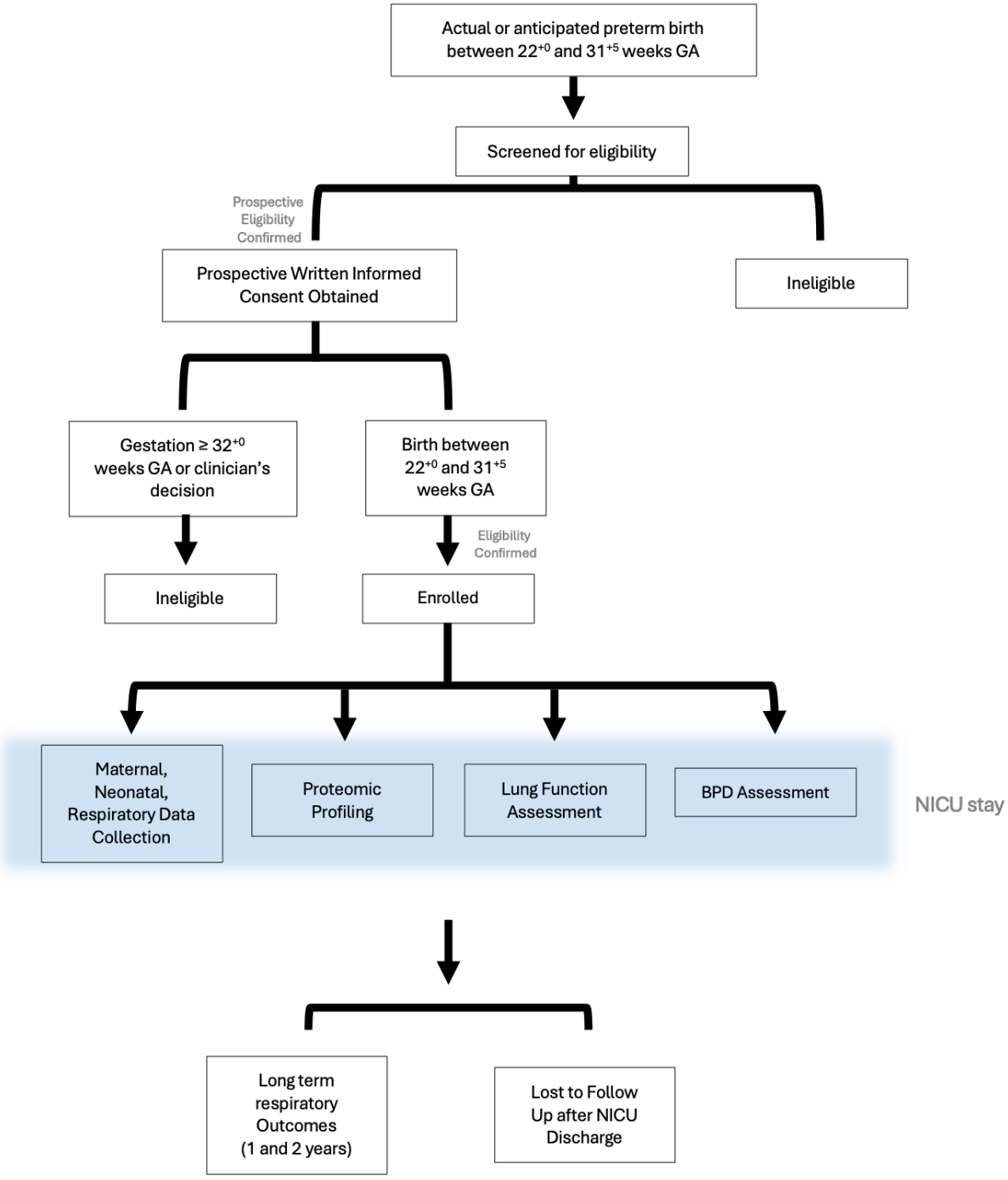

Figure 4a Summary of Study Schema.

See Table 5a for details of schedule of assessments and data collection at each assessment.

4.2 Overall design

This is a prospective observational study involving infants born preterm who require care in a participating Victorian Level 6 NICU. The overall purpose is to generate a large dataset to robustly define the different early life preterm respiratory phenotypes, and then interrogate that dataset to determine the relationship between these phenotypes (and biomarkers used to define them) and later BPD and 1- and 2-year respiratory health status.

Infants born between 22+0 and 31+6 weeks' gestation who meet all inclusion (and no exclusion) criteria and provide prospective written informed parental/guardian consent will be included in this study. This gestational age (GA) range has been chosen to align with those included in the Australian and New Zealand Neonatal Network (ANZNN) database. The ANZNN database aims to capture all preterm infants born in Australia and New Zealand (opt out consent).

The BLUEPRINT study expects to enrol 550 infants over the course of the 3-year enrolment period across 5 tertiary level NICUs in Victoria.

Longitudinal observations and measurements will be taken from plasma proteins, LUS and EIT, in addition to a standardised set of clinical measured designed to reflect physiological status, delivered clinical care and known antenatal, maternal and postnatal respiratory outcome risk factors. All participating infants will receive standard NICU care as per the treating institution.

Eligible infants will be consented prospectively, and eligibility confirmed prior to study enrolment. Prior to blood sampling and lung imaging, the parents and/or legal representative of the infant will be reminded of their participation in the BLUEPRINT study. Prior to discharge, patient contact details will be confirmed by the study team for follow-up study timepoints. Post-discharge follow-up at 1 and 2 years of corrected gestational age is not mandatory but encouraged. Follow-up assessments will be completed at the MCRI (50 Flemington Rd, Parkville 3052).

4.3 Study population

4.3.1 Eligibility Criteria

Infants born between 22+0 and 31+6 weeks' gestation admitted to a participating centre who meet all the inclusion criteria and none of the exclusion criteria are eligible for enrolment on the BLUEPRINT study.

4.3.2 Inclusion Criteria

Each infant must meet all inclusion criteria to be eligible for enrolment on this study:

1. Born between 22+0 and 31+6 weeks' gestation.
2. Birth admission in a participating perinatal Victorian NICU.
3. The infant has a parent/legal representative capable of understanding the informed consent document and providing consent on the infant's behalf AND consent is obtained antenatally or within 72 hours from birth.

4.3.3 Exclusion Criteria

If any of the following exclusion criteria are met, the infant will be ineligible for enrolment on this study:

1. Infants with a known major congenital anomaly (such as oesophageal atresia and central nervous system anomalies), congenital cardiac disease, congenital diaphragmatic hernia, congenital lung abnormalities and/or pulmonary hypoplasia.
2. Pulmonary hypoplasia due to anhydramnios **or** oligohydramnios before 22 weeks in which the neonatal clinician anticipates that pulmonary hypoplasia related respiratory failure will be the major respiratory problem in early postnatal life.
3. Primary cause of admission unrelated to prematurity.
4. Refusal of informed consent by their legally acceptable representative.
5. The infant does not have a parent/legal representative who can provide informed consent or consent cannot be obtained before 72 hours of age.

In addition, any infant identified by the treating medical team may be excluded from the study at the request of the treating clinician in consultation with the site lead investigator.

4.4 Recruitment of potential infants

4.4.1 Recruitment planning

Any infant born into a participating perinatal Victorian NICU between 22+0 and 31+6 weeks' gestation will be eligible to participate in the BLUEPRINT study if they meet all the inclusion and none of the exclusion criteria (as described in section 4.3.1). The study design is an observational longitudinal prospective study for which prospective consent will be obtained for all enrolled infants. Eligible infants will be screened as per section 4.5.2.

Infants will be asked to attend a follow-up respiratory and development assessment at 1 and 2 years of corrected age. All follow-up assessments will be conducted at the MCRI. Whilst follow-up assessments are not mandatory, they are strongly encouraged. The MCRI BLUEPRINT study team will contact the family prior to their scheduled follow-up assessment timepoint as a reminder of their participation in the BLUEPRINT study. Birthday cards will be sent by the study team on behalf of the BLUEPRINT study on the infant's first and second birthdays.

4.4.2 Stages of recruitment

Screening: Potentially eligible infants will be identified from admission from the participating NICUs, antenatal and inpatient ward lists by the site study investigators and research nurses at each site.

Screening Log: A record of all eligible infants will be maintained in a dedicated REDCap screening database and updated by a member of the study team.

Enrolment: Once a potentially eligible infant (antenatally or postnatally [before 72 hours of age]) has been identified, a member of the study team will first determine a suitable time to meet with the parent(s)/legal guardian. This will be done in consultation with the treating clinical staff to ensure the psychological circumstances and stressors of the parent/legal guardian, as well as the clinical events and situation, are considered.

At a suitable time, a member of the study team will meet with the parent(s)/legal guardian in the antenatal wards or NICU (or via telephone or digital communications platforms if access restrictions are in place) to obtain consent (see Section 4.5). If informed consent has been provided and the infant still meets eligibility criteria at birth, the infant will be enrolled into the study and allocated a unique study code.

If the mother carries beyond 31+6 weeks' gestation, or consent is given after 72 hours from the infant's birth, the infant will no longer be eligible for enrolment and any signed consent form will be void. The signed consent form will be maintained by the site staff and archived as per the study protocol.

Concurrent Research Enrolment: The BLUEPRINT study encourages and supports concurrent enrolment with other clinical research studies unless the co-enrolment compromises the integrity of BLUEPRINT outcomes. Concurrent study enrolment will enhance recruitment efforts at participating sites, and potentially allow streamlining of data collection to reduce participant burden. Any patient who is identified as eligible for enrolment on multiple research studies, should be discussed and enrolled at the discretion of the Site Principal Investigator. Should further discussion be required, the Co-ordinating Principal Investigator should be contacted prior to enrolling the infant on BLUEPRINT. Enrolment of BLUEPRINT participants on other research studies or clinical trials should be documented in the participant's medical record and in REDCap.

The policy on Concurrent Research Enrolment is as follows:

1. All trials and studies that likely involve participants who are also eligible for the BLUEPRINT study must be discussed with the Principal Investigator and Co-ordinating Principal Investigator, and the protocols shared with the study team, prior to co-enrolling any infants.

2. All such studies may or may not be compatible with the BLUEPRINT study, and a full discussion will be undertaken with all parties to resolve potential for co-enrolment.

3. Enrolment of BLUEPRINT participants in concurrent studies that involve consent for the child and family must be documented within the participant's medical record and on the Final NICU CRF within the BLUEPRINT REDCap Data Management System (DMS).

4.5 Consent

The investigator or delegated member of the study team will discuss the study with the infant's parent/legal guardian either in the antenatal period or within the first 72 hours after birth. The investigator will provide the Participant Information and Consent Form (PICF) to the parent/legal guardian. This document will describe the purpose of the study, the procedures to be followed, biospecimen sample collection requirements and the risks and benefits of participation.

The investigator will check that the parent/legal guardian comprehends the information provided and answer any questions about the study. The parent/legal guardian will be invited to provide written consent. Consent will be voluntary and free from coercion.

The investigator who conducted the consent discussion will also sign the informed consent form and it will be documented in the infant's medical record that consent has been provided. When all the inclusion/exclusion criteria have been addressed and the eligibility of the infant confirmed, the infant is considered enrolled. The signed consent form will be filed in the infant's shadow folder maintained at each participating site. A copy of the consent form will also be given to the parent/legal guardian.

Written consent will be primarily sought. If the parent/legal guardian is unable to be approached in person, verbal consent will be sought over the telephone or via teleconference. Digital recording of tele-consent will not occur. However, documentation of the parent/legal guardian's contact details, outcome of verbal consent and follow up will be documented in the "Record of Verbal or Virtual Consent" form within the study's REDCap (Research Electronic Data Capture) screening log. If verbal or virtual consent is obtained, the investigators will follow up with the parent/legal guardian for written consent at the next available opportunity. Having a newly-born preterm baby in the NICU is stressful. The timing for follow up of written consent will be coordinated with the treating clinical team to ensure minimal distress or inconvenience for the parents/guardians.

Regular contact, in the form of yearly newsletters and birthday cards, will be made with the families leading up to the 2-year assessment.

Documentation in the patient's medical record will be made for any consent (verbal, virtual or written) related to this study as per institutional standard practice.

5. STUDY SCHEDULE OF ASSESSMENTS

5.1 Study timeline

The study will be conducted over a 5-year period from the date the first infant is enrolled. Screening and prospective eligibility will be assessed antenatally by a delegated member of the study team. Prospective consent will be obtained by the infant's parent/legal guardian and can be obtained up until 72 hours of life (prior to plasma sampling). Eligibility will be confirmed prior to enrolment and documented as per the manual of operating procedures. Infants enrolled in the study will undergo assessments as outlined in the Schedule of Assessments (section 5.2) and summarised in Table 5a. Follow-up assessments will occur at 1 and 2 years corrected gestational age. Infants will participate no longer than 3 months after they turn 2 years of corrected age. In addition, relevant maternal/perinatal data, and adverse events (AEs) and other safety events/risks will be collected, based on maternal interviews, extracted from medical charts, and entered in the study REDCap database.

5.2 Schedule of assessments

Table 5a Study Timeline and Schedule of Assessments

Schedule of Primary Assessments											**Follow-up Assessments**	
Assessment/ Procedure	**Screening**	**Baseline (Birth)**	**24h post-birth**	**48h post-birth**	**72h (±12h) post-birth**	**Day 7 (±12h)**	**Day 14 (±1d)**	**Day 28 (±1d)**	**36wks PMA (+7d)**	**End of NICU Stay**	**Year 1 (corrected age)**	**Year 2 (corrected age)**
Informed Consent and Documentation of Eligibility	X[#]											
Maternal data collection^		X										
Neonatal and Respiratory data collection^		X	X	X	X	X	X	X	X	X		
Lung Function Assessment (LUS and EIT)			(X)	(X)	X	X	X	X	X		X	X
Proteomic profiling (Plasma Sampling)					X	X						
BPD Assessment*									X			
Clinical Health Assessment											X[1]	X[1]
Respiratory Follow-up (Questionnaire)											(X)	(X)
Development Assessment (PARCA-R)											(X	(X)
Adverse Event Reporting			X	X	X	X	X	X	X			

(X) denotes optional assessments
[#] Consent is permitted to be obtained up until 72 hours of life, prior to plasma sampling
^ Clinical data and Perinatal Medical History will be collected from Patient Medical Records
*BPD Diagnosis and Severity assessed according to the Walsh and Jensen criteria
[1] Clinical Health Assessment to be performed if patient returns in-person for FU timepoints

6. STUDY VISITS AND PROCEDURES

6.1 Description of procedures

Eligible infants for whom consent have been obtained will be studied. Prior to lung imaging or plasma sampling measurement at 72 hours and 7 days after birth, a member of the study team will approach the clinical team (medical or nursing staff) to re-confirm suitability to participate in the study. This will be repeated prior to lung imaging measures at Days 14, 28 and time of BPD diagnosis.

The parent/legal guardian will be contacted by a member of the study team prior to assessment at Days 14, 28 and time of BPD diagnosis, and again for the 1 and 2 year follow-up assessments to ensure that they are still aware of their infant's participation in the BLUEPRINT study and provide an opportunity to ask any questions or withdraw from the study, as per the manual of operating procedures.

6.2 Notes on study visits

6.2.1 Screening

Screening data will ascertain whether an infant is eligible for the study, the process of consent and outcome will be documented in the infant's medical record. Screening will occur prior to an infant's birth or up to 72 hours after birth as detailed in Section 4.4 – *Recruitment of Potential Infants*. Each site will maintain a screening log of all screened mothers and infants, indicating who is eligible and who is not, and of eligible mothers who have consented to the study and who have refused study participation and why (if known).

Screening information (i.e. the Screening Log) will be recorded and maintained directly within a BLUEPRINT REDCap Data Management System (DMS). Note: this Screening Database is maintained separate to the main BLUEPRINT study database. Refer to the BLUEPRINT CRF Completion Guidelines for further information regarding data entry.

The following **Eligibility Data** will be documented within the infant's medical record, prior to transcription into BLUEPRINT REDCap database:

- Confirmation that the infant meets the eligibility criteria for the study (i.e. all inclusion and no exclusion criteria)
- Documentation of Participant Identification Number (PID) assigned.

6.2.2 Standard study visits

Screening
• Documentation of Consent and Eligibility status

Baseline (Birth)
• Maternal Data Collection • Neonatal and Respiratory Data Collection

24h and 48h post-birth
• Neonatal and Respiratory Data Collection • *Lung function assessment (LUS and EIT) (optional)*

72h post-birth
• Neonatal and Respiratory Data Collection • Lung function assessment (LUS and EIT) ±12h • Plasma Collection (proteomic profiling)

Day 7
• Neonatal and Respiratory Data Collection • Lung function assessment (LUS and EIT) ±12h • Plasma Collection (proteomic profiling)

Day 14 and Day 28
• Neonatal and Respiratory Data Collection • Lung function assessment (LUS and EIT) ±24h

36 weeks' PMA
• Neonatal and Respiratory Data Collection • BPD Assessment (Walsh, Jensen criteria)

End of NICU
• Neonatal and Respiratory Data Collection

Follow-Up; Year 1 and Year 2
• Respiratory Follow-up • Lung function assessment (LUS and EIT) • Developmental Follow-up (PARCA-R)

6.2.3 Clinical Data

The following data will be collected by a member of the study team from the participating infant's and their mother's medical files/records, investigative reports, and bedside monitors:

Antepartum Data: These data will be completed, and entered directly into the BLUEPRINT REDCap DMS at a suitable time following infant enrolment, including:

- Documentation of mode of consent
- Confirmation that the infant meets the eligibility criteria for the study (i.e., all of the inclusion and none of the exclusion criteria)
- Documentation of Participant Identification Number (PID#)
- Maternal medical history
- Medication history
- Demographic information of the mother and infant, including:
    - Mother's year of birth
    - Mother's race/ethnicity (if consented to provide)
    - Infant's full date of birth, time of birth, sex
- Pregnancy data, including:
    - Gestational age (in weeks/days)
    - Birth weight (g)
    - Plurality of the pregnancy
    - Mode of delivery and reason
    - Fetal Growth Restriction
    - Reason for preterm birth
    - Documentation of the use of any antenatal steroids, or other emergency antenatal/perinatal medication
    - Documentation of any adverse events and/or safety issues

Delivery Room and initial NICU Stabilisation: A delegated member of the study team will enter the following data directly into the BLUEPRINT REDCap DMS at a suitable time following infant enrolment, including:

- Time of delivery/birth
- Use of delayed cord clamping and timing
- Delivered positive end-expiratory pressure settings/level; highest and lowest and duration
- Positive Inflating Pressure levels (if used)
- Fraction of inspired oxygen (FiO_2) delivered; lowest and highest levels and duration
- Duration of delivery room stabilisation
- Intubation status
- Heart Rate (HR); lowest reading
- Oxygen Saturation (SpO_2); lowest and highest levels
- Highest modified oxygenation index (SpO_2/ FiO_2) (calculated post hoc)
- Documentation of other delivery room interventions and/or medications used
- Final respiratory settings on departure from delivery room

First week of NICU care after birth: A delegated member of the study team will enter data directly into the BLUEPRINT REDCap DMS relating to the clinical course during the first 24, 48, 72 hours and at 7 days of life, including:

- Use of supplemental oxygen, including highest level of FiO_2 in the first 24, 48, 72 hours and 7 days of life
- Details of respiratory support including type of support and duration (including none, low flow and high flow nasal cannula, continuous positive applied pressure and other forms of non-invasive and invasive ventilation modalities)
- Highest pressure, flow, delivered volume settings as appropriate for all types of respiratory support used during the first 24, 48, 72 hours and 7 days of life
- Intubation and extubation events up to Day 7
- Survival status/date of death (during hospital stay, if applicable) / reason for death
- Documentation of any adverse events and/or safety issues
- Relevant respiratory and other treatments or outcomes, including:
    - Use of postnatal steroids, including types, timing and accumulated doses
    - Use, type, delivery method and dose of exogenous surfactant
    - Use of caffeine or other respiratory stimulants
    - Use of inotropes, including type and accumulated dose
    - Need for fluid or blood product bolus therapy
    - Haematological and biochemical investigation results
    - Use of antibiotics and proven culture positive results
    - Incidences of significant brain injury (IVH, periventricular leukomalacia), pneumothorax and pulmonary interstitial emphysema
    - Patent ductus arteriosus (PDA) and any associated treatment
    - Incidences of other clinical events or diagnoses that may impact respiratory status
- Details of blood sample collection
- LUS data including quality and number of images and LUS score
- EIT data including quality and number of images, belt size used and regional ventilation measures (centre of ventilation, %Tidal volume per region and inhomogeneity index)

6.2.4 Lung Function Assessments: Lung Imaging

A LUS and EIT manual of procedures and operations will be developed for each Site. LUS and EIT imaging will be conducted at 72 hours and on Day 7 after birth (with a ± 12-hour window) at a time deemed clinically suitable by the bedside nursing and/or medical team. Where possible, both imaging modalities will be performed concurrently. If clinically appropriate to do so, EIT and LUS images will also be collected at 24 and 48 hours after birth as additional data. Data on the tolerance of lung imaging modalities will be documented. Factoring in time to allow the infant to settle between care, any position changes and image acquisition, between 30-60 minutes is anticipated to record all imaging required at each visit.

6.2.4.1 Electrical Impedance Tomography (EIT)

EIT will be used to measure regional tidal ventilation and aeration patterns using the LuMon EIT system (Sentec AG, Landquart, Switzerland), a standalone monitor. The Neonatal Research Group at the MCRI have been using the Sentec EIT system since 2014 and is familiar with all aspects of use[22, 28-31]. The Sentec EIT system integrates image reconstruction models to known characteristics of the infant chest utilising a customised electrode EIT belt system. EIT signals will be sampled at 48Hz and recorded directly into the hard drive capacity of the EIT hardware (stored as standard of care by date and time within the EIT bedside system and not identifiable patient features). A trained technician from the MCRI Neonatal Research Group will provide the necessary training and equipment (hardware and consumables) at each participating site.

The EIT system measures the regional changes in lung volumes through weak alternating currents applied to the body. These travel along the paths of least resistance through the chest, thereby creating electric potentials at the body surface. These potentials are being measured continuously by electrodes and turned into tomographic images of lung function. The electrodes are contained in a belt-like disposable textile patient interface that is fastened to the infant's thorax (**Figure 6a**).[32, 33] The Sentec EIT electrode system includes a belt made with a knitted polyamide 6 filament yarn trilobal silver electrode fabric (approved for human use). This fabric surrounds a 0.25 cm deep and 1-2 cm wide soft medical grade padding (to ensure the belt can be applied snuggly around the chest to avoid movement, and yet not interfere with normal tidal chest movement). To ensure optimal electrical conductance without the need for adhesive gel electrodes, the belts are lightly coated with standard (warmed) ultrasound gel and then wrapped around the chest at nipple level and secured with a Velcro fastener.

The EIT belt is in sterile packaging and can be left in situ for up to 72 hours and suitable for single use only. Infant handling time during belt application is <1min. PI Professor Tingay was involved in the development of this belt system,(32, 33) and has used this EIT belt system on more than 100 infants at the Royal Children's and Royal Women's Hospitals without complication.

The Sentec EIT system (and components) are CE marked as a Class IIa medical device in the European Unit and was approved by the TGA as a Class IIa device for all age groups (including neonates of all gestations) on 5 October 2022 (ARTG entry 396994). The Sentec EIT system will not be used to alter clinical care or influence clinical decision making in this study.

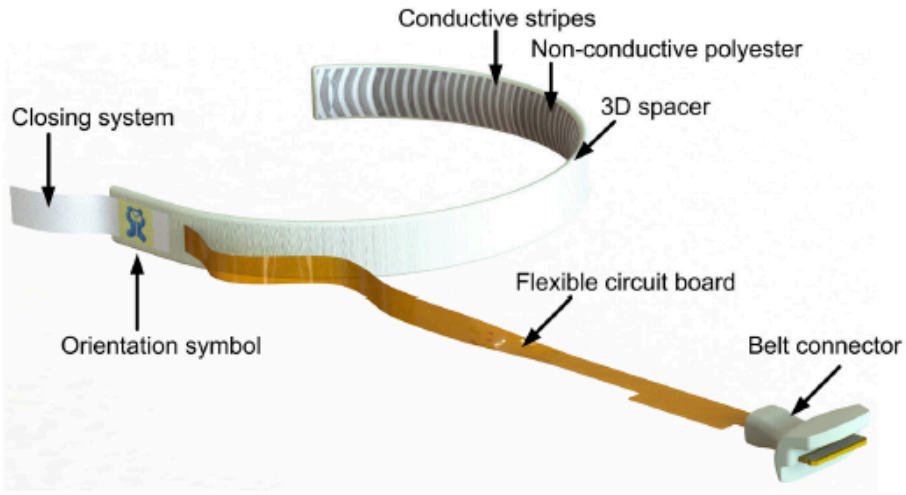

Figure 6a: The EIT sensor belt. Neonatal electrode belt consists of a striped electrically conductive textile, a 3D space fabric, an ultra-flexible printed circuit board and a Velcro closing system. Each of the 32 electrodes has a size of 1cm. Reproduced from Ref #(33)

The EIT belts are available in various sizes based on GA and body weight. In the case of uncertainty, the lower sized belt will be used. The belt will be coated with warmed ultrasound gel and placed around the infant's chest (at nipple level) by gently lifting the infant (or lower part) off the bed and placing the belt under the thorax or sliding the belt under the infant using the soft Velcro tag as a lead. This process takes approximately 10-20 seconds to complete and involves no greater risk than routine clinical nursing cares; if possible, it will be placed at the same time as clinical care.

An example of an EIT belt on an infant is shown in **Figure 6b**. Once in place, the wearing of the EIT belt does not interfere with any aspect of the usual clinical process. The only scenarios in which the belt may

impact usual clinical practice is when the region of the infant's chest encompassed by the belt needs to be accessed in an emergency. This includes for the provision of chest compressions and/or insertion of a chest tube or needle thoracentesis. In both cases, the EIT belt Velcro can be undone, and the chest exposed in <2-5 seconds and would not restrict emergency therapies.

As soon as the EIT belt Velcro is secured, the EIT monitor display provides sensor quality feedback via a traffic light system display of red (bad), orange (adequate) and green (good). If the EIT belt has more than 8 of the 32 sensors indicated with red light, it will require re-adjustment or additional contact gel. If readjusting belt position does not interrupt clinical care, the team will do so. If it would interrupt clinical care, EIT recordings will not be made, and the belt removed at a time that suits the clinical team.

Once adequate/good signal contact is confirmed, EIT data will be recorded continuously (at 48 frames per second) for 2-5 minutes (if there is unacceptable signal interference or artefact, then the measurement will be repeated if practical). A total of 4 EIT images will be repeated over 2-5 minutes recording blocks. At the end of this period, the measurements will cease and the EIT belt Velcro unfastened and removed at a time that will be least invasive for the infant. EIT imaging will be performed in the supine position to a standardised gravity-dependent plane for comparisons. If the clinical team intend to re-position the infant immediately before or after an EIT recording, additional recordings in prone or lateral position will be made. If the clinical team request EIT monitoring for clinical needs, the system and belt will be kept in place until not required anymore.

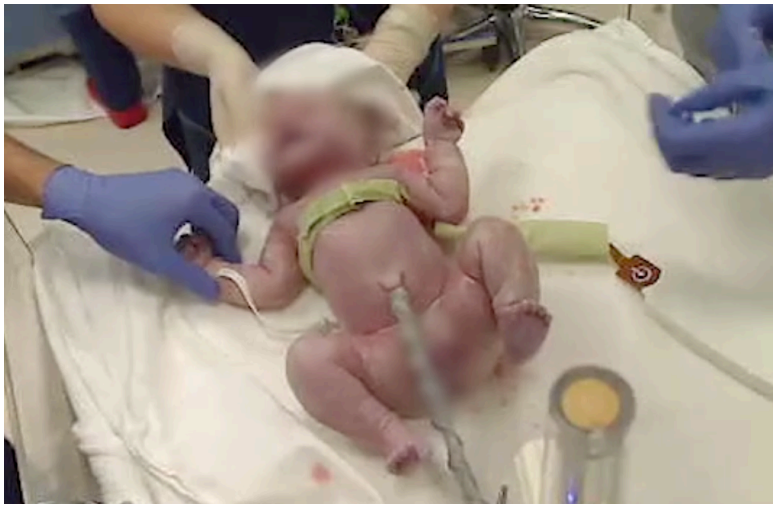

Figure 6b An EIT belt in situ on an infant during resuscitative management at birth. EIT belt does not impact provision of clinical care. Reproduced with permission of parents from Ref # (22).

The following links provide a video summary of this process and how the use of EIT monitoring is quick, simple, non-invasive and does not interrupt clinical care (note: these videos are during resuscitative care at birth in a delivery room setting):

https://doi.org/10.26188/19430372

https://doi.org/10.26188/14058608

6.2.4.2 Lung Ultrasound (LUS

LUS images will be obtained in the supine position using a GE Venue Go, Venue 50 (GE Healthcare, USA), and a Philips Affinity ultrasound system (Philips, Netherlands) with a "hockey stick," L8-18i linear transducer set at a depth of 2.5cm and a gain of 55 decibels. The focus will be adjusted to be positioned at the pleural line. All filters will be deactivated. Video clips of 3 seconds will be acquired and stored under the allocated study identification number. If the infant is deemed unstable and not able to tolerate ultrasound imaging by the imaging team, the investigators will return at a more suitable time. The infant will be handled for <5 minutes for each LUS assessment.

6.2.4.3 Image acquisition

6 images in total will be acquired per ultrasound scan. This will comprise of the upper and lower anterior, and lower lateral regions. M-mode will be performed with each image. The ultrasound transducer will be orientated longitudinally to capture 2-3 intercostal spaces according to infant size.

6.2.4.4 Imaging procedure

If the EIT belt is in situ, it will be unfastened to allow LUS imaging without further handling. Once the infant is settled, LUS scans will be acquired. The probe will be gently placed on the infant's chest and ultrasound gel (warmed immediately prior to scanning) will be used to ensure sufficient probe contact. Images will be acquired as per section 5. LUS image acquisition will take approximately 5 minutes.

6.2.4.5 Infant handling and safety during LUS and EIT

There will be no excessive handling associated with the ultrasounds or EIT required for this study. Infants will only be repositioned for image acquisition if it is deemed appropriate by the treating clinician and the bedside nurse midwife. If the infant is deemed clinically unstable for data acquisition, the procedure will be deferred. The site investigators will work closely with the treating clinician and bedside nurse/midwife to ensure the safe acquisition of ultrasound and images. Disruption to routine care will be minimised by coordinating data acquisition with clinical care.

6.2.4.6 Image coding

EIT Images

All EIT images will follow a standardised labelling nomenclature in the following format:

BLPRNT_[Study ID]_[Time point (DAYxx)]_[sequence order]

Time points will be recorded using the following labels:

Time Point	Label
72 Hours	DAY3
Day 7 (168 hours)	DAY7
36 Weeks PMA	36WK

Example: An infant (Study ID: 1234) receives a lung ultrasound at 72 hours (Day 3).

Therefore, the third imaging period will be labelled as: **BLPRNT_1234_DAY3_3**

LUS images

All LUS images will follow a standardised labelling nomenclature in the following format:

BLPRNT_[Study ID]_[Time point (DAYxx)]_[lung_region]

Regions must be labelled as the following:

Region	Label
Right anterior upper	RAU
Right anterior lower	RAL
Right lateral lower	RLL
Left anterior upper	LAU
Left anterior lower	LAL
Left lateral lower	LLL

Example: An infant (Study ID: 1234) receives a lung ultrasound on Day 3. The image from the right anterior upper region will be labelled as: **BLPRNT_1234_DAY3_RAU**

6.2.5 Blood Sampling and plasma isolation

Details of blood sampling and processing methodology are outlined in the BLUEPRINT Lab Processing Manual.

A minimum sample volume of 0.2ml of whole blood is required at each timepoint to perform the mass spectrometry analysis. Whole blood samples (0.5ml) will be collected into EDTA microtubes by a research or bedside nurse via either umbilical arterial line or capillary access. The required 0.2 - 0.5 ml of whole blood is consistent with the volume of blood required for common clinical investigations in preterm infants (approximately 0.3-0.5 mL for electrolyte or full blood count analysis) and represents between 0.17-0.5% of circulating blood volume (500-1500 g weight).

Blood samples will be collected at 72 hours (±12 hours) and Day 7 (±12 hours) post-birth from all enrolled infants. Where possible, arterial sampling will be used as this is less invasive for the infant. Capillary sampling involves breeching the skin (heel) using a micro-lancet. Micro-lancet capillary sampling is common practice in all Victorian NICUs and considered standard of care when arterial access is not available. Both arterial and capillary sampling will be conducted in accordance with current NICU blood sampling guidelines at participating sites. The ± 12 hours sampling window allows for the impact of blood sampling on the infant to be limited. Capillary blood samples will be matched to routine clinical sampling timelines to ensure there are no additional skin breeches associated with participation in the project. If routine sampling is not occurring within the allowable ±12-hour window, a protocol deviation will be noted in the RedCap database. All blood samples will be performed by the bedside nurse/midwife, phlebotomist or doctor who will be instructed not to persist with sampling if the procedure would expose the infant to longer distress or pain. To further ensure infant safety, a member of the study team will confirm with the clinical team the suitability of sample collection prior to each

collection based on the individual characteristics of the infant. A sample will only be taken if the treating clinician is satisfied the infant would not be harmed.

Following collection, the samples will be labelled by the bedside/research nurse in accordance with the BLUEPRINT Study Proteomics Manual of Operating Procedures and Lab Processing Manuals and forwarded to the hospital Pathology Department for processing prior to transfer to the MCRI.

Prior to mass spectrometry analysis, one plasma aliquot will be transferred to the Baker Research Institute (BRI; Professor D Greening) and immediately stored in a secure freezer at -80°C. The plasma sample barcode will be used by a MCRI research staff member to log the sample "transferred to BRI" using the secure OpenSpecimen platform. Post mass spectrometry, remnant plasma samples (including empty vials) will be transferred back to MCRI and logged as "returned to MCRI". Mass spectrometry data files will use the de-identified barcode, with all data files uploaded to the secure server at MCRI for later analysis and incorporation in the study database. Any excess plasma will be stored at -80°C in secured and monitored freezers allocated to the Neonatal Research Group (MCRI). Any residual plasma samples will be securely stored at -80°C for a period of 10 years to facilitate potential future validation of markers identified by the Neonatal Research Group at MCRI.

6.2.6 Day 14 and Day 28 Assessment

Previous studies of LUS to predict BPD status have suggested that Day 14 and Day 28 are the most useful time points.(21) LUS and EIT images will be conducted on Day 14 and Day 28 after birth (with a ± 24-hour window) as per 72 hours and Day 7 methods (see Section 6.2.4). Suitability to obtain imaging data and best timing will be re-affirmed with the clinical team. Where feasible, the parent/legal guardian will be notified of upcoming assessments as per the manual of operating procedures. In addition, the following clinical data will be collected:

- Type of respiratory support and use of supplemental oxygen at midday on Day 14 and Day 28
- All types and duration of respiratory support used between Days 8-14 and Days 15-28
- Intubation and extubation events since Day 7
- Survival status/date of death (during hospital stay, if applicable) / reason for death
- Documentation of any adverse events and/or safety issues
- Relevant respiratory and other treatments or outcomes, including:
  - Use of postnatal steroids, including types, timing and accumulated doses
  - Use, type, delivery method and dose of exogenous surfactant
  - Use of caffeine or other respiratory stimulants
  - Use of inotropes, including types, timing and accumulated doses
  - Use of pulmonary hypertensive treatments, including types, timing and accumulated doses
  - Use of antibiotics and proven culture positive results
  - Incidences of significant brain injury (IVH, periventricular leukomalacia), pneumothorax and pulmonary interstitial emphysema, necrotising enterocolitis and retinopathy of prematurity
  - Patent ductus arteriosus (PDA) and any associated treatment
  - Incidences of other clinical events or diagnoses that may impact respiratory status
- LUS data including quality and number of images and LUS score
- EIT data including quality and number of images, belt size used and regional ventilation measures (centre of ventilation, %Tidal volume per region and inhomogeneity index)

6.2.7 36-weeks' PMA BPD Assessment

BPD status during 36 weeks' PMA will be assessed using the Modified Walsh definition and standard oxygen reduction test (ORT), and Jensen criteria for Grading from no BPD, mild, moderate and severe (Grade 0-3) (5, 10). Death before 36 weeks' PMA due to a primary respiratory cause will be coded as severe BPD using the 2018 NICHD Grade 3A BPD definition; *'early death between 14 days postnatal age and 36 weeks' PMA from persistent parenchymal lung disease and respiratory failure that is not attributable to other neonatal morbidities'*. Inclusion of death due to non-respiratory causes may result in misclassification in the prediction models. The primary and critical contributors to all deaths before 36 weeks' PMA will be documented to allow secondary analysis for 'all cause' mortality in the prediction models. The 36-week BPD Assessment should occur on the date determined to be 36+0 to 36+6 weeks PMA.

If the BPD assessment is completed outside of the 36+0 to 36+6 weeks' PMA assessment window, as the infant was discharged home from hospital early self-ventilating in air (SVIA) before 36+0 weeks PMA, then these infants will be coded as not having BPD. Should the condition of the infant change within the 36-weeks PMA (e.g. they are re-admitted to any hospital between discharge home and 36+6 weeks PMA), then the participating site must update the 36-Week PMA BPD Assessment CRF, if applicable.

Lung imaging will be repeated as per Section 6.2.4, and the appropriateness of studying the infant will be re-affirmed with the clinical staff. For scheduled weekly or pre-discharge measures, consent will also be re-affirmed with the parents/legal representative and documented in the participant's medical record as per the Manual of Operating Procedures.

6.2.8 NICU Stay

A delegated member of the study team will record data relating to the clinical course after Day 28, and then relevant secondary outcomes during NICU stay until hospital discharge, death, withdrawal, or 44 weeks' PMA (whatever comes first) for each infant; including:

- Survival status/date of death (during hospital stay, if applicable) / reason for death
- Type and duration of respiratory support and use of supplemental oxygen during stay not already documented, specifically:
    - Need for invasive respiratory support between birth and 36 weeks' PMA
    - Age at and duration of invasive respiratory support between birth and 36 weeks' PMA and discharge
    - Age at and duration of non-invasive respiratory support between birth and 36 weeks' PMA and discharge
    - Age at and duration of all modes of respiratory support between birth and 36 weeks' PMA and discharge
    - Age at and duration of supplementary oxygen between birth and 36 weeks' PMA and discharge
- Oxygen requirement at discharge
- Intubation and extubation events since Day 28
- Growth parameters
- Documentation of any adverse events and/or safety issues
- Relevant respiratory and other treatments or outcomes, including:
    - Use of postnatal steroids, including types, timing and accumulated doses
    - Use, type, delivery method and dose of exogenous surfactant
    - Use of caffeine or other respiratory stimulants

- Use of inotropes, including types, timing and accumulated doses
- Use of pulmonary hypertensive treatments, including types, timing and accumulated doses
- Use of antibiotics and proven culture positive results
- Incidences of significant brain injury (IVH, periventricular leukomalacia), pneumothorax and pulmonary interstitial emphysema, airway abnormalities or obstruction (such as sub-glottic stenosis), PDA requiring treatment, surgical procedures, necrotising enterocolitis, and retinopathy of prematurity
- Incidences of other clinical events or diagnoses that may impact respiratory status

In addition, relevant baseline maternal, antenatal, and demographic data not collected prior to Day 7 will be recorded before discharge. Consent to include families in ongoing study newsletters and other dissemination of study information will be re-affirmed during this period. Families will also be asked to confirm willingness to participate in 1- and 2-year follow-up and consent to collect contact information (phone number and email) and Medicare details. Families that agree to follow up will be provided with reminder information.

6.2.9 1Year and 2 Year corrected age Follow-Up Visit Data

A follow-up visit will occur at 1 year (12-months' corrected age ± 1 month) and 2 years (24-months' PMA ± 2 months) to assess for respiratory and general health outcomes. Children will be invited to attend the MCRI Research Clinic. The Research Coordinator/Study Team, or other appropriately trained staff, at the MCRI will record data relating to:

1. Mortality before 1 year and at 2 years corrected age
2. General health and well-being since last assessment, including hospital presentations and medications
3. Weight, length and head circumference
4. Respiratory events since last assessment, including hospital presentations/admissions, confirmed respiratory tract infections, wheezing episodes requiring treatment, use of steroid and/or bronchodilator treatments.
5. Smoking exposure and burden (self-reported by parent)
6. Duration of oxygen therapy after discharge (if applicable)
7. Postcode at time of assessment (smoking and postcode are needed for analysis of potential confounders, such as pollution exposure and socioeconomic class)
8. Documentation of general gross motor ability (including diagnosis of cerebral palsy) and cognitive ability, any visual impairment or blindness, any hearing impairment or deafness
9. PARCA-R Questionnaire (developmental assessment questionnaire completed by parents), if applicable

LUS and EIT imaging will be performed as per scheduled assessments during the NICU stay.

If families are unable to attend in-person for follow-up assessments, a telephone consultation questionnaire with parent/legal guardian or an online health status questionnaire (via REDCap) will be offered. In instances when a telephone consultation cannot be performed, a Direct-to-Parent Questionnaire will be forwarded to parents/legal guardians for completion (if consent provided), which

includes a brief health assessment and a validated child development assessment aimed at parents (PARCA-R Questionnaire; Dev Med Child Neurol 20 04; 46:389–97). The PARCA-R Questionnaire is a complete questionnaire that can be used to assess children's cognitive and language development at 2 years of age.

The Direct-to-Parent questionnaire will be administered via a web-based survey located on the secure REDCap server at MCRI, in Melbourne, Australia. The child-specific link to the Direct-to-Parent questionnaire, and any alerts/reminders disseminated, will be sent electronically to parents. All communication efforts will be documented in the child's medical record or shadow file.

6.3 Infant/child withdrawals and losses to follow up

6.3.1 Withdrawal of consent

The parent/legal guardian is free to withdraw their infant from the study at any time upon request. Withdrawing from the study will not affect their or their infant's relationship with, or care by, the hospital and affiliated healthcare professionals. A dedicated CRF page in REDCap will be used to capture the date of withdrawal of consent and, if provided, the reason, noting that participants themselves do not need to provide a specific reason for withdrawing. Upon notification of a study withdrawal, the study team should complete the 'End of Study' CRF within the BLUEPRINT REDCap DMS which will include reasons for withdrawal.

Withdrawal from the study does not indicate refusal to use existing data already collected unless the parent/legal guardian specifically informs the study team that they withdraw consent for the collection of their infant's medical, imaging and plasma data. Following withdrawal, subsequent data will only be collected if explicit permission is provided by the parent/legal guardian.

Any infant may be permanently discontinued from study participation for any of the following reasons listed below:

- They are found to have conditions listed in the exclusion criteria during the course of the study
- Withdrawal of consent from study at any time by their parent/legal guardian
- Investigator determines it is in the best interest of the infant

In these cases, the infant will either be replaced in the study cohort or moved into the follow-up phase of the study until withdrawal of consent, death or study close out.

The procedures following the withdrawal or discontinuation of a study infant are outlined in the Manual of Operating Procedures.

Every effort should be made to obtain information on infants who withdraw from the study. An excessive rate of withdrawals can render the study uninterpretable; therefore, unnecessary withdrawal of infants by parent/legal guardian should be avoided. Should a parent/legal guardian decide to withdraw their infant, all efforts will be made to complete and report the observations as thoroughly as possible.

6.3.2 Lost to follow-up

An infant will be considered lost to follow-up if they fail to return for the 1- or 2-year scheduled visits and is unable to be contacted by the study team.

The following actions must be taken if an infant fails to return to the clinic for a required study visit:

- The site will attempt to contact the parent/legal guardian of the infant and reschedule the missed 1- or 2-year follow-up visit and counsel the parent/legal guardian on the importance of maintaining the study visit schedule and ascertain if their infant should continue in the study.
- Before an infant is deemed lost to follow-up, the Site PI or designee will make every effort to regain contact with the infant's parent/legal guardian. Where possible, a minimum of 3 attempts to contact the family will be documented and may include telephone call and/or email and, if necessary, a certified letter to the parent/legal guardian's last known mailing address or local equivalent methods.

Should they continue to be unreachable, they will be considered to have withdrawn from the study, with a primary reason of lost to follow-up.

6.3.3 Replacements

Infants who are withdrawn from the study by a parent/legal guardian will only be replaced in situations where:

- The infant is subsequently identified as having conditions listed in the exclusion criteria (see Section 4.4.3)

6.3.4 Study Closure

An infant is considered to have completed the study if they have completed all study visits (2-year visit assessment if still alive) as outlined in the Schedule of Assessments.

The study may be extended in duration if recruitment has not proceeded at the expected rate.

The end of the study is defined as completion of the last visit or procedure shown in the Schedule of Assessments in the study at all study sites has occurred and all queries have been answered and data entered ('database locked'). At this stage, the Coordinating PI will ensure that all HRECs and RGOs as well as all regulatory and funding bodies have been notified with an End of Study declaration submitted.

Decisions and recommendation on study closure may be made by:

- BLUEPRINT Data Safety Monitoring Committee (DSMC), or
- Study Sponsor

7 POTENTIAL RISKS RELATED TO STUDY CONDUCT

7.1 Risks to the safety and rights of the study participants

Safety and adverse events will be monitored during the study to ensure timely detection of events that may affect safety or continued participation.

Blood sampling is a common practice in the NICU and low risk. There will be no additional risk related to blood sampling than those associated with routine practice with this procedure. These include the potential risk of infection from capillary and arterial sampling, and pain or discomfort from the use of a

microlancet during capillary sampling. Standard site practices will be followed for all blood sampling and research sampling will be aligned with clinical sampling to minimise the risk exposure. The BLUEPRINT protocol has been designed to utilise proteomic analysis which allows us to yield maximum amount of data from small amounts of blood. The volume of blood required for this research study is very small (0.5ml per sample, two samples per patient) and adds very little to the volume of blood taken from preterm infants as part of their routine care. Many preterm infants may require a blood transfusion during their NICU care, the small volume of blood required for this study is unlikely to contribute any additional risk that the participant will require a blood transfusion or not.

There will be minimal to no excessive handling associated with the LUS and EIT recordings required for this study. Infants will only be repositioned for image acquisition if it is deemed appropriate by the treating clinician and the bedside nurse. The investigators will work closely with the treating clinician and bedside nurse to ensure the safe acquisition of LUS and EIT images. If the infant is deemed too unstable then the infant will not be studied.

7.1.1 Definitions

For this study, the following safety definitions will be observed.

Adverse Event (AE): Any untoward medical occurrence in a human subject, including any abnormal sign (for example, abnormal physical exam or laboratory finding), symptom, or disease, temporally associated with the subject's participation in research, whether or not considered related to the subject's participation in the research.

7.1.2 Specific Protocol-Defined Adverse Events and Serious Adverse Events

It is anticipated that there will be minimal risks involved to the infants and families. However, the BLUEPRINT study population involves critically ill preterm infants that are anticipated to have a high number of AEs. Therefore, **the protocol has designated study-specific AEs that are required to be reported within a certain timeframe.** Table 7a below lists specific protocol-defined AEs and the timeframe within which they are to be reported.

Whilst there will be minimal handling associated with LUS and EIT records, study-specific AEs will be reported by the Investigator and assessed for the severity and relatedness. The potential risk of breeches in skin integrity represents a reportable protocol-defined adverse event, and specific data on skin integrity will be included in the study database, as will questions regarding tolerance of the imaging. Clinical staff and parents will be notified of any potential adverse events related to the study (including breeches of skin integrity).

Table 7a Specific Protocol-Defined AEs:

Adverse Event Definition	Reporting Time Frame
Oxygen requirement of FiO_2 ≥20% of baseline for 2 hours or more after any imaging measurement	Within **72 hours after** each scheduled lung function assessment
Breech of skin integrity or skin irritation associated with LUS or EIT	Within **24 hours** of each scheduled lung function assessment
Apnoea requiring stimulation or other intervention that required the EIT or LUS scan to be terminated	Within **72 hours** after each scheduled lung function assessment
Acute oxygen desaturation (SpO_2 <90%) that required the EIT or LUS scan to be terminated	Within **72 hours after** each scheduled lung function assessment

Proven infection (culture or PCR positive)	Within **72 hours** of a scheduled assessment involving lung function assessments or plasma measurements

The AEs outlined in Table 7a occurring in each specified time frame are to be recorded on the AE Form within the BLUEPRINT Study REDCap DMS

Any additional adverse events deemed **related or possibly related** to study assessments or procedures, as evaluated by the site PI, should also be documented on the AE form, and entered in the DMS.

EIT belts will only be allocated to a single infant and cleaned and sterilised (UV) using local site standard operating procedure between imaging assessments to minimise the risk of infection. Cables and hardware will be cleaned as per site operating practices between use. Belts that are damaged, worn or soiled will be replaced.

7.2 Assessing the Severity of an Infant's AE

The Severity (mild, moderate, severe, life threatening or fatal) of an AE will be assessed by an investigator, with the following exception:

- Hospitalisation/extended hospitalisation due to progression of clinical condition will not be considered an AE for the purposes of this study.

7.3 Assessing the Relatedness (Causality) of an Infant's AE to Study Procedures

All adverse events/serious adverse events must have their relationship to the study procedures assessed by the Site PI who evaluates the adverse event based on temporal relationship and clinical judgment. The degree of certainty about causality will be determined using the categories below. In a clinical study, the assessments should always be suspected as potentially contributing to an AE.

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

Degree of Causality	Causal Relationship		Description
1	Unrelated	Unrelated	The AE is clearly NOT related to study procedures
2	Unlikely		The AE is doubtfully related to the study procedures
3	Possible	Related	The AE may be related to the study procedures
4	Probable		The AE is likely related to the study procedures
5	Definite		The AE is clearly related to the study procedures

Preterm birth and subsequent NICU care are associated with many risks including severe morbidity and mortality. Many of these risks overlap with events that would or could be an AE/S due to the study assessments.

7.4 Assessing the Severity of an Infant's AE

The Site PI will be responsible for assessing the severity of an adverse event (AE). The determination of severity for all adverse events should be made by the investigator based upon medical judgment and the severity categories of Grade 1 to 5 as defined below.

Grade	Severity	Description
Grade 1	Mild	Asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated
Grade 2	Moderate	Moderate; minimal, local, or non-invasive intervention indicated; limiting age-appropriate instrumental activities of daily living (ADL)
Grade 3	Severe	Severe or medically significant but not immediately life-threatening; hospitalisation or prolongation of hospitalisation indicated; disabling; limiting self-care ADL
Grade 4	Life Threatening	Life-threatening consequences: urgent intervention indicated
Grade 5	Fatal	Death related to AE

7.5 Assessing the Expectedness of AEs

An AE that is deemed to be related to study procedures must be assessed to determine whether the event is expected or unexpected in terms of the current known safety profile and risks associated with the study procedure. An AE will be considered unexpected if the nature, severity, or frequency of the event is not consistent with the risk information previously described for the study procedure.

Expected adverse reactions are AEs that are known to occur for the study procedures. Expectedness is assessed based on the awareness of AEs previously observed, not on the basis of what might be anticipated from the properties of the study protocol.

It is the responsibility of the BLUEPRINT Medical Monitors to assess expectedness of all reported AEs for the study.

7.6 Documentations of AEs

For the purposes of this study, the Site PI is responsible for recording all AEs, with the following exceptions:

- Conditions that are present at screening and do not deteriorate will not be considered adverse events.

Adverse events will be reported on the study REDCap Adverse Event Form, the details of each AE will include:

- A description of the AE
- The onset date, duration, date of resolution
- Severity (mild, moderate, severe, life threatening or fatal)

- Any action taken, (e.g., none, any treatment given, follow-up tests administered)
- The outcome (i.e. recovering/resolving, not recovered/not resolved, recovered/resolved, recovered/resolved with sequelae, fatal, unknown)
- The likelihood of the relationship of the AE to the study procedure (unrelated, unlikely to be related, possibly related, probably related, definitely related).

Changes in the severity of an AE will be reported as separate events. AEs characterised as intermittent will be documented for each episode. All AEs will be followed to adequate resolution, where possible.

The BLUEPRINT Manual of Operating Procedures details how to record adverse events.

7.7 Capturing and Eliciting Adverse Event Information

7.7.1 Adverse Event Reporting

All AEs must be recorded from the time the participant is enrolled until 48 hours after the study primary endpoint.

The specific protocol-defined AEs (refer to Tables 7a) occurring in each specified time frame are to be recorded on the AE CRF within the BLUEPRINT study DMS.

7.7.2 Coordinating Principal-Investigator Reporting Procedures

The Coordinating Principal-Investigator (CPI) (or delegate) is responsible for:

- Implementing and maintaining a suitable recording system to record information from all AEs received from participating sites.
- Ensuring that the CPI is notified of each AE to enable the AE to be assessed by the CPI and any other appropriate reviewers for nature (expected/unexpected), and causality.
- Reporting AEs to ethics committees according to applicable local laws.
- Considering information provided by (non-serious) adverse event data.
- Informing each participating site of new information arising from serious and non-serious adverse events that may affect the conduct of the Study, or the rights, interests, safety, or wellbeing of study patients.
- Providing any updated safety information to all Site PIs.

The Coordinating Principal-Investigator (or delegate) is also responsible for providing the following additional safety information to the approving HRECs:

1. Provide an annual safety report, including a summary of the evolving safety profile of the study.

7.7.3 Potential Risks Related to Study Conduct

Risks to the successful conduct of the study are low. The study has obtained NHMRC-funding to conduct the study to the 2-year follow-up endpoint.

1. **Recruitment:** Sites have active and established neonatal research programs with extensive experience in recruiting families of different ethnicities and sociodemographic backgrounds during times of stress associated with preterm birth. Many preterm infants are eligible for multiple unrelated studies, we have established cooperative systems at all sites of approaching families to minimise their burden, whilst maintaining high recruitment rates.

2. **Attrition at 2-year follow up:** From experience, these families are highly motivated. We will maximise follow-up rates using proven strategies which include contact by experienced staff who have known the families from birth (often involved in recruitment), having multiple alternative contacts in addition to parents, social media, and maintaining up-to-date contact details through regular contact with families via newsletters and birthday cards. We will consider the need for multiple imputation to account for missing data, if necessary.

3. **Delays in ethics approvals and multisite agreements:** We are familiar with the local HREC processes and staff. Ethics submissions will be prioritised to ensure timely processing.

4. **Death before a Scheduled Assessment:** If a participating infant passes away, then data already collected will be used, however, parents will not be asked to participate in any subsequent assessments or data collection. The research coordinators will check the infant's tertiary hospital medical records prior to contacting families to arrange follow-up appointments to ensure, where possible, that the current status of an infant is known, particularly for deceased infants.

8 DATA AND INFORMATION MANAGEMENT

8.1 Overview

The Study Investigators are responsible for storing essential study documents relevant to data management and maintaining a site-specific record of the location(s) of the site's data management-related Essential Documents.

Site Principal Investigators are responsible for maintaining adequate and accurate source documents that include all key observations on all participating infants at their site. Source data will be attributable, legible (including any changes or corrections), contemporaneous, original, accurate, complete, consistent, enduring and available. Changes to source data (hardcopy and electronic) must be traceable, must not obscure the original entry, and must be explained where this is necessary. A site-specific **Source Document Plan** will be maintained to indicate the location(s) of source documents.

The Site PI will also maintain accurate data collection forms (also known as case report forms - CRFs) and be responsible for ensuring that the collected and reported data is accurate, legible, complete, entered into the study database in a timely manner and enduring. To maintain the integrity of the data, any changes to data (hardcopy and electronic) must be traceable, must not obscure the original entry, and must be explained where this is necessary.

Any person delegated to collect data, perform data entry or sign for data completeness will be recorded on the delegation log and will be trained to perform these study-related duties and functions.

Full details of all processes are provided in a separate study-level **Data Management Plan**.

8.2 Data management

8.2.1 Data generation (source data)

In this study, the following types of data will be collected:

- Personal identifying information (names, dates of birth, contact details, postcode, URN/MRN).
- Sensitive information including health data relating to the antenatal and postnatal (NICU) period from both infant and mother.
- Respiratory and general health status at 1 and 2 years of age (including details of hospital and health care service presentations between initial NICU (birth) admission and 2 years.
- Plasma samples collected at 72 hours and 7 days.
- LUS and EIT imaging data as detailed in schedule of assessments table.

8.2.2 Source Document Plan

The source documents for this study include the participating sites' electronic/paper medical record, questionnaires completed by the infant's parent/legal guardian and/or researcher (paper); recorded data from automated instruments, laboratory reports and the signed parent/legal guardian information and consent forms. Each site participating in the study will maintain a site-specific **Source Document Plan** that will document the source, i.e. original recording, for each data discrete item/category of items collected for the study. This Source Document Plan, signed and dated by the PI, will be prepared prior to recruitment of the first infant and will be filed in the site's Investigator Site File.

8.2.3 Data capture methods and data use, storage, access, and disclosure during the study

The Data Coordinating Centre (DCCe) provides a REDCap DMS for the secure entry and storage of BLUEPRINT data. REDCap (Vanderbilt University, Nashville, TN) is a secure, web-based software platform designed to support data capture for research studies, providing:

1. an intuitive interface for validated data capture;
2. audit trails for tracking data manipulation and export procedures;
3. automated export procedures for seamless data downloads to common statistical packages; and
4. procedures for data integration and interoperability with external sources (34).

Reasons for non-participation will be documented. Access to the BLUEPRINT REDCap database will be granted to individual participating site users by the DCCe Data Manager or Central Study Coordinator.

Participating site clinical teams/study teams will enter study data directly into the secure BLUEPRINT study DMS.

Designated and authorised participating site staff must complete the CRFs and supporting documentation for each infant within a timely manner of each assessment occurring. All staff delegated by the Site PI to enter data must be indicated on the Site Signature and Delegation of Authority Log.

Study related essential documents maintained for the study will be filed within the BLUEPRINT electronic Study Master File (eTMF) platform, Florence eBinders™, a cloud-based SaaS software maintained by Florence Healthcare, hosted in Germany, EU via Amazon Web-Services (AWS) and backed up daily.

Investigator Site Files (ISF) pertaining to each participating site will also be maintained electronically via the Florence eBinders™ platform by each participating site, to enable remote monitoring of essential study and regulatory documentation.

Full information on the data variables is in the Study Data Management Plan.

8.3 Image reporting

8.3.1 Lung ultrasound aeration score

Ultrasound images will be reported using our scoring system that has been validated against gold standard measures of lung volume (Figure 8a).[35]

This score ranges from 0-5 (0; worst aeration, 5; best aeration). The total scores for LUS images will range from 0-30 (categorical ordinal variable).

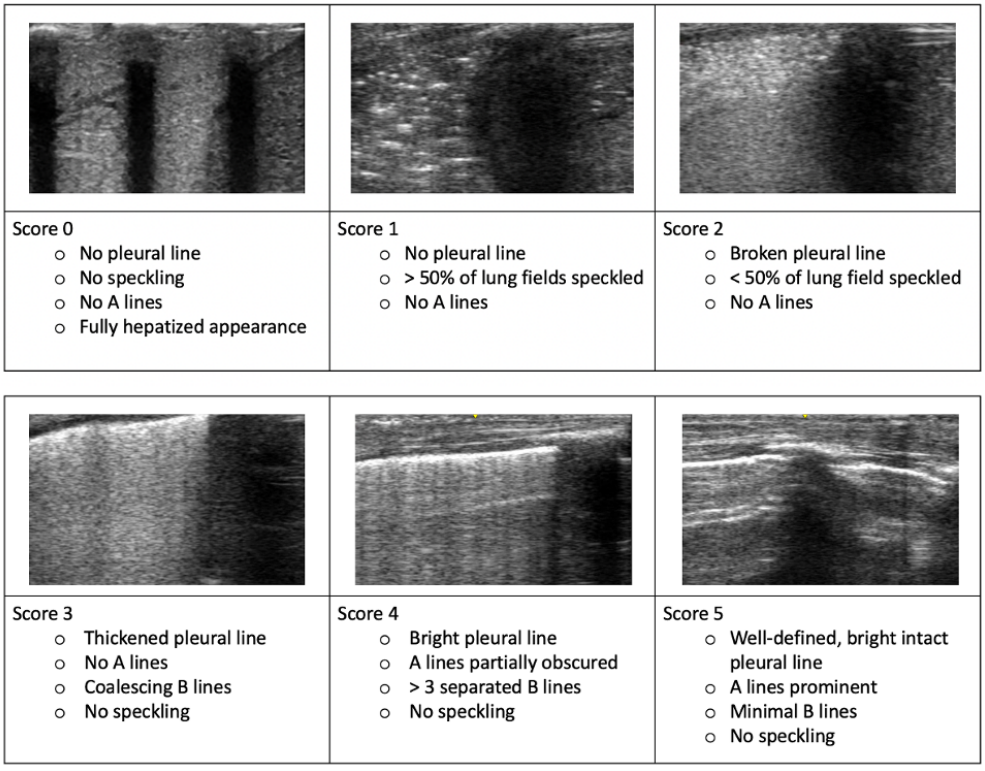

Figure 8a Lung ultrasound scoring system.

8.3.2 Reporting and storage of ultrasound images

All ultrasound images will be acquired by the participating site investigators. Data and ultrasound images will be de-identified. Ultrasound images will be reported at two timepoints;

1) at the time of image acquisition by the ultrasound operator and

2) 1 month post image acquisition by an investigator who is blinded to the clinical details of the patient.

All ultrasound images will be transferred from ultrasound systems using a password protected hard drive which will be kept in a secure location at each site. Locally stored images will be coded using the participants Study ID only. Images will then be uploaded to the BLUEPRINT REDCap DMS, within the infant's corresponding CRF. Images will be regularly deleted from the participating sites hard drive once successful upload to the BLUEPRINT REDCap DMS is confirmed.

8.3.3 Reporting and storage of EIT data

All EIT images will be acquired by the participating site investigators. Raw EIT files will be de-identified. At the time of image acquisition, the operator will complete the "EIT Image Quality CRF" in the BLUEPRINT REDCap DMS.

All raw EIT files will be transferred from the EIT system using a password protected hard drive which will be kept in a secure location at each participating site. Locally stored raw EIT files will be coded using the infant's Study ID only. Images deemed artefact-free by a trained Site Operator will be analysed using the Sentec lfvReader (a simple software package which automatically extracts EIT outcome measures for every included breath/inflation as an xml file).

These data, along with the raw EIT files, will then be uploaded to the BLUEPRINT REDCap DMS within the infant's corresponding CRF. EIT data will be regularly deleted from the participating sites hard drive once successful upload to the REDCap servers is confirmed.

EIT files deemed to contain artefact will be uploaded to the BLUEPRINT REDCap DMS without analysis. Automated notifications in the "EIT Image REDCap CRF" will notify the study team at the MCRI that images have been uploaded. These raw EIT files will then be analysed manually by an experienced operator (supervised by PI Tingay) for artefact cleaning and analysis within IbEX (a more sophisticated EIT software analysis package that allows manual breath and artefact exclusion). A random selection of raw EIT files deemed artefact free will be selected from each participating site during the study conduct period for analysis within IbEX for quality assurance.

8.3.4 Site investigator image acquisition competency

A member of the study team at each participating site who has completed the Certificate in Clinician Performed Ultrasound (CCPU) for Neonatal Lung ultrasound will be nominated to lead the training of image acquisition competency and maintenance of skills. This training and accreditation has been provided through the Australian Society of Ultrasound in Medicine (ASUM). Any additional investigators will need to demonstrate image acquisition competency prior to performing ultrasounds for this study. This will comprise of 5 lung ultrasounds. Image quality, labelling, storage and competency will be assessed by suitably qualified members of the BLUEPRINT study team. Multimedia teaching packages will be provided to each site for acquisition, processing and storage of EIT data. These will complement site training by the PI team, which will include competency assessments (which will be stored as a log for each Site).

8.4 Use of the data

The data will be used for the analyses specified in the protocol and Statistical Analysis Plan (SAP).

Following the completion and analysis of the study, the data will be retained long-term following the mandatory archive period for use in future research projects.

8.5 Storage and access

Hard copy data will be stored by the participating sites in a locked cabinet in a secure location, accessible to the study team only.

Electronic data will be securely stored in MCRI's REDCap database system and in files stored in MCRI's network file servers, which are backed up nightly. **Files containing private or confidential data will be stored in a secure location accessible only by delegated personnel.**

REDCap is hosted on MCRI infrastructure and is subject to the same security and backup regimen as other systems (e.g. the network file servers). Data is backed up nightly to a local backup server, with a monthly backup taken to tape and stored offsite. REDCap maintains an audit trail of data created/updated and deleted events that is accessible to project users who are granted permission to view it. Access to REDCap will be provided via an MCRI user account or (for external collaborators) via a REDCap user account created by the MCRI system administrator. The permissions granted to each user within each REDCap project will be controlled by, and will be the responsibility of, the study team delegated this task by the PI. REDCap has functionality that makes adding and removing users and managing user permissions straightforward. All data transmissions between users and the REDCap server are encrypted. The instructions for data entry to REDCap must be read and the training log signed prior to personnel commencing data entry on REDCap.

Authorised representatives of the sponsoring institution as well as representatives from the HREC, RGO and regulatory agencies may inspect all documents and records required to be maintained by the Investigator for the participants in this study. The study site will permit access to such records.

Refer to the BLUEPRINT Data Management Plan (DMP) for detailed information on data storage.

8.6 Disclosure

The study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study, or the data will be released to any unauthorised third party without prior written approval of the sponsoring institution. Clinical information will not be released without written permission of the infant's parent/legal guardian, except as necessary for monitoring by the HREC, RGO or if required by law.

9 SAMPLE MANAGEMENT

9.1 Specimen & Biobanking

9.1.1 Registration of a Biobank

A biobank (named "BLUEPRINT" Biobank) will be established and allocated to the Neonatal Research Group at MCRI to securely store de-identified plasma samples derived from patients recruited within the BLUEPRINT study (2x aliquots per time-point) for a 10-year period (Biobank Registration Form [BRF] submitted for approval 28/02/2024). The BLUEPRINT Biobank will be located at the Murdoch Children's Research Institute, within the MCRI Freezer facility (ground floor of Royal Children's Hospital), requiring restricted card access to enter the facility. CI Dr Prue Pereira-Fantini is the allocated custodian responsible for the BLUEPRINT Biobank.

Blood samples will only be collected from enrolled infants whose parent/legal guardian have provided written informed consent for the collection of biosamples. For each infant, 2x blood samples (0.2-0.5mL) will be collected at timepoints as defined by the protocol, at 72 h (± 12h) and 7days (± 12h).

Whole blood will be processed by local site pathology departments to obtain 2x plasma aliquots per time-point, which will then be transferred into de-identified barcoded microtubes that will be stored in the BLUEPRINT Biobank.

Samples will be de-identified using a barcode system, and sample storage, distribution and destruction will be tracked using the OpenSpecimen platform (http://openspec.mcri.edu.au). Researchers performing data analysis on plasma samples (proteomic and statistical analyses) will be required to use de-identified data only. Samples will be re-identifiable with restricted access permitted to CI Prue Pereira-Fantini, Data Management Specialist Richard Hall or delegated research staff members by the BLUEPRINT Study Coordinating Centre.

Access into the MCRI -80°C freezer facility is by swipe card only, which will limit user access. The allocated freezer for long-term storage of BLUEPRINT plasma samples is equipped with the following system controls;

- **Building Management System Alarms:** If the temperature increases >-70°C, an alarm is triggered to the main Royal Children's Hospital console, whereby facility controllers will check the freezer to ensure temperature integrity is maintained. Emergency contact (CI Prue Pereira-Fantini) listed on the freezer will be notified if required.
- **Independent Temperature Sensing System (ITSS):** which are secondary temperature probes that communicate directly to the Melbourne Children's Bioresource Centre (MCBC) using a wireless network to notify of temperature drops.

Samples will be destroyed if an infant's parent/legal guardian requests to withdraw the infant from the study, the biobank or consent request expires, or if the biobank is discontinued. Samples will be destroyed in line with biobank standard operating procedures at the central MCRI site. A destruction record will be maintained on OpenSpecimen as part of sample tracking.

9.1.2 Sample storage and management

The blood processing and storage protocol outlined in **Figure 8b** has been developed in collaboration with Associate Professor David Greening (BRI) and has been optimised to facilitate the extraction of proteins from plasma for mass spectrometry analysis. Blood processing and storage will be in accordance with the BLUEPRINT Manual of Procedures and Lab Processing Manuals.

A summary of sample storage and management is provided in Figure 8b.

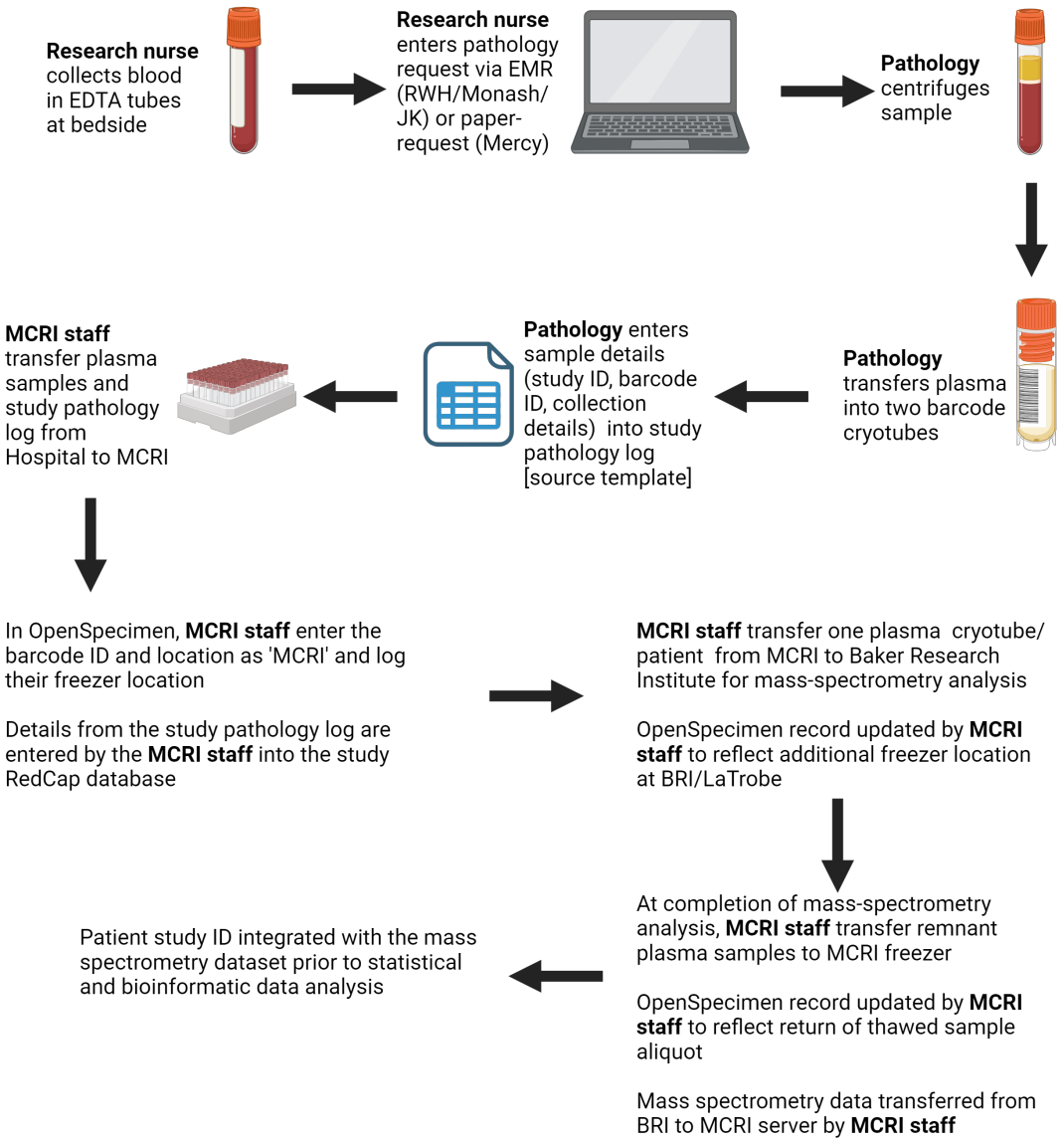

Figure 8b: Plasma sample collection, storage and management protocol with responsible parties highlighted in bold.

9.2 Data confidentiality

Infant confidentiality is strictly held in trust by the PI, participating investigators, research staff, and the sponsoring institution and their agents. This confidentiality is extended to cover testing of biological samples and genetic tests in addition to the clinical information relating to participating participants.

To preserve confidentiality and reduce the risk of identification during collection, analysis and storage of data and information, the following will be undertaken:

(1) The number of sensitive/confidential variables collected for each infant has been minimised. The data collected will be limited to that required to address the primary and secondary objectives.

(2) Infant identifiers will be stored separately to the data collected; documents with identifiers will be stored separately to infant data. Infant data and samples will be identified through use of a unique participant study number/code assigned to the study infant ("re-identifiable"). Plasma samples will be bar-coded with a unique barcode that will be linked to the unique infant study number/code via REDCap. The Site PI is responsible for the storage of a master-file of names and other identifiable data with the infant ID; access to this document will be restricted to the site study team and authorised persons as listed previously. The master file should be stored securely, and separately, from study data in locked/password-protected databases with passwords kept separately. Due to the study population and study aims, GA at birth, sex and age will need to be documented in the study CRFs (GA and sex are major determinants of respiratory status after preterm birth, and age is needed to coordinate scheduled assessments).

(3) Separation of the roles responsible for management of identifiers and those responsible for analysing content. The data will be analysed by the statistician, who will be provided with anonymised data identified only by the unique infant study ID.

9.3 Quality assurance

The Data Coordinating Centre (DCCe) will review completed data for accuracy, completeness, and consistency, whereby requests for corrections and/or clarification of data (e.g., data queries) will be sent to the Site PI or designated participating site staff when inconsistencies are identified during the review process.

All corrections and alterations to CRF data must be addressed by participating sites in a timely manner and according to instructions provided within the BLUEPRINT CRF Completion Guidelines. Additions, deletions and/or alteration to data within the CRFs will be recorded in a secure, computer-generated, time-stamped audit trail, including the reason for the change.

Please refer to the BLUEPRINT CRF Completion Guidelines for further details regarding data entry and query management.

9.4 Archiving - Data and document retention

The data collected as part of this study will be retained for 7 years after the last infant turns 18 (e.g. 25 years) in line with NHMRC guidelines.

It is the responsibility of the Coordinating Principal-Investigator (CPI) to inform the Site Principal Investigators and Participating site teams when these documents no longer need to be retained. Study documents are not permitted to be destroyed without the prior written agreement from the CPI. Should Principal Investigators wish to assign the study records to another party or move them to another location prior to this time, advanced notice must be provided to the CPI, in writing. Paper data will be shredded and securely disposed, and computer and video files will be deleted.

A zipped folder containing a copy of participating sites Investigator Site Files (ISF) maintained electronically via the Sponsor's approved platform, Florence eBinders™, will be provided to each participating site by the DCCe at the time of archiving. Participating sites will also have the option of downloading an entire copy of their ISF and storing a copy locally on institutional servers.

9.5 Long-term Custodianship

Infants in the BLUEPRINT study may be able to participate in future follow-up studies into later childhood or beyond. As such, after the archive period, the data will be anonymised for preservation to reduce the risk of re-identification in accordance with MCRI policy and protocols. The Study CPI (Prof Tingay, or delegate) will be the long-term custodian following the archive period.

9.6 Data sharing

The following will be made available at 3 months after completion of analysis and article publication for long-term use by future researchers from a recognised research institution whose proposed use of the data has been ethically reviewed and approved by an independent committee and who accept MCRI's conditions for access:

- Individual infant data that underlie the results reported in this article after de-identification (text, tables, figures and appendices)
- Study protocol, Statistical Analysis Plan, PICF

10 STUDY OVERSIGHT

10.1 Governance structure

The organisational and governance structure is summarised in Figure 9a. The BLUEPRINT Study Research Network will form the Study Steering Committee (SSC). This consists of the Coordinating Principal Investigator and Associate Investigators across 5 sites in Victoria, the MCRI and Consumer Representatives involved in the NHMRC CTCS Grant application, plus additional local members and international advisors.

The SSC with the study statistician will provide the scientific leadership for the study. The Study Coordinating Centre (MCRI) and the Data Coordinating Centre (DCCe; Study Coordinating Centre and Clinical Epidemiology and Biostatistics Unit [CEBU]) based at the MCRI, are collaboratively managing the study.

The Study Coordinating Centre is responsible for clinical leadership, research operations and overall study management and the DCCe is responsible for statistical analysis and database management.

The Medical Monitors will provide overall safety oversight for the study by reviewing all reported SAEs in real-time, determining the expectedness and relatedness of the safety event and the appropriate course of action. If deemed necessary, the Medical Monitors, will report any significant safety issues (SSIs) identified to the Data and Safety Monitoring Committee (DSMC).

The Study Coordinating Centre (c/o BLUEPRINT Study Coordinator) will serve as the liaison between the participating sites, the SSC and all other committees supporting the study.

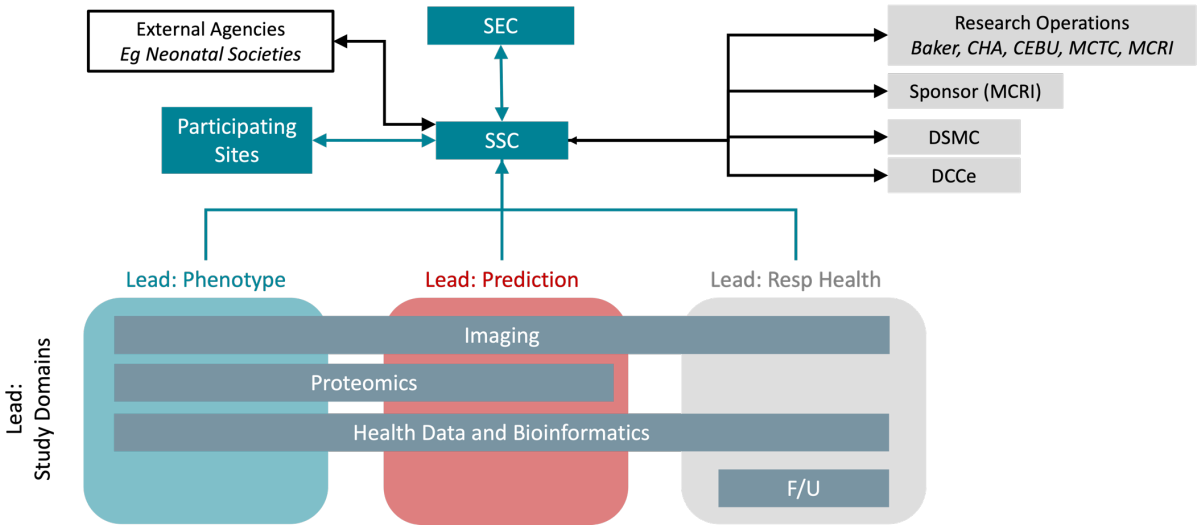

Figure 10a Overview of BLUEPRINT Study Organisational Structure.

Abbreviations: SEC; Study Executive Committee, SSC; Study Steering Committee, DSMC; Data and Safety Monitoring Committee, DCCe; Data Coordinating Centre, CHA; Centre of Health Analytics; CEBU, Clinical Epidemiology and Biostatistics Unit; F/U, Follow-Up.

10.1.1 Study Steering Committee (SSC)

A Study Steering Committee (SSC) will be established to provide expert advice, leadership and overall supervision and ensure that the study is conducted to the required standards. This includes being responsible for overall study direction and coordination of subcommittees and taskforces. The SSC will meet at least quarterly, with more frequent meetings as needed, and will work to a Terms of Reference. SSC membership will include at least 1 representative from each of the participating sites in the BLUEPRINT study.

Within the SSC there will be a **Study Executive Committee (SEC).** The SEC will consist of the study leadership group (Study Coordinating Centre and CCe) and study statistician, and be responsible for all scientific, fiscal, and administrative decisions on behalf of the study. The SEC will meet fortnightly during the development and site start-up phase of the study and may reduce to less frequent meetings during the study conduct and follow-up phases.

Within the SSC there will also be a Lead Investigator identified for the 3 principal study goals of the BLUEPRINT study:

1) Early Life Respiratory Phenotype,

2) Prediction modelling of respiratory outcomes and

3) Respiratory Health Follow-Up.

The Lead Investigator of each study goals will not be the PI (Prof Tingay or delegate) and will report directly to the PI Investigator and update the SEC and SSC at each meeting.

Specific SSC sub-groups will be formed to provide expert advice, efficiency and direction of key aspects of the BLUEPRINT study, and to develop procedures, training material and analysis plans for the specific

interests of each group (under consultation with the SEC, Study Coordinating Centre and DCCe). These include subgroups that will address the following study domains:

1. Lung Imaging
2. Plasma biomarker/proteomics
3. Data management and integration
4. Prediction modelling
5. Follow-Up

Each Study Coordinating Centre subgroup will meet at least quarterly, with more frequent meetings as needed, and will work to a Terms of Reference. Each Study Coordinating Centre Subgroup will be led by one member of the SSC with the provision to include external membership if approved by the Study Coordinating Centre. Meetings will be minuted, and a report presented to the SSC after each meeting for approval. A member of the SEC will attend each Study Coordinating Centre subgroup as an advisor. Each Study Coordinating Centre will report to SEC and also meet regularly with the Phenotype, Prediction and Respiratory Health SEC leads as required.

The SSC will have the option to appoint appropriately qualified external advisors to the BLUEPRINT study as needed. External advisors will be appointed by SSC majority.

10.1.2 Independent Data and Safety Monitoring Committee (DSMC)

An Independent Data Safety Monitoring Committee (DSMC) will be established to review emerging external evidence and monitor protocol compliance, AEs/SAEs, significant safety issues (SSIs), mortality and progress of recruitment in accordance with the BLUEPRINT DSMC Charter.

The SSC will provide the DSMC with a charter. The DSMC will receive both blinded and unblinded interim safety reports and will consist of the following members:

- A team of clinicians/neonatologists
- A neonatal ethicist
- An independent statistician
- Additional members as required.

Refer to the BLUEPRINT DSMC Charter for further details.

10.1.3 Study Coordinating Centre

The MCRI will be the Study Coordinating Centre and provide central coordination of study operations across all participating sites as well as operational support and oversight in areas such as: infant recruitment, study operating procedure development, data management and protocol adherence. The Study Coordinating Centre will be directed by Prof Tingay, with overall study support and management provided by Dr M Pang, and report directly to the SSC and SEC. The Study Coordinating Centre will function to:

1. Direct the clinical aspects of protocol development and implementation.
2. Develop training materials and instructions for the intervention algorithms and other clinical procedures.
3. Oversee study governance.
4. Oversee the BLUEPRINT study network of supporting committees.
5. Coordinate participating site start-up activities and site staff training and implementation (with each Site Lead).

6. Coordinate the development and distribution of all aspects of study protocol.
7. Overall study oversight and management.

The Study Coordinating Centre has employed a Study Coordinator who will assist in these tasks and work closely with the DCCe Team. A member of the Study Coordinating Centre will conduct all site initiation meetings/visits and annual site monitoring visits.

10.1.4 Data Coordinating Centre (DCCe)

A DCCe will be established to support the Study Coordinating Centre and research network to assure collaboration across sites, along with standardisation and uniformity of procedures, to yield high-quality data. The DCCe will be coordinated by an appropriately qualified Data Scientist. Specific responsibilities include:

1. Providing statistical leadership data management support for the conduct of the study.
2. Develop and implement the data management systems.
3. Establish and maintain data collection and entry procedures.
4. Train and monitor participating sites in data collection and data integrity.
5. Participate in all study meetings and collaborate with the Study Coordinating Centre and SSC in preparation of publications and presentations.

10.1.5 Site Study Management Group (SMG)

The Site PI is responsible for supervising any individual or party to whom they have delegated tasks at the study site. They must provide continuous supervision and documentation of their oversight. To meet this GCP requirement, a small group will be responsible for the day-to-day management of the study and will include at a minimum the Site PI and project manager/research nurse/study coordinator. The group will closely review all aspects of the conduct and progress of the study, ensuring that there is a forum for identifying and addressing issues. Meetings must be minuted with attendees listed, pertinent emails retained, and phone calls documented.

10.2 Quality management, assurance, and control

The Study PI has responsibilities for quality management and will build quality assurance (QA) into the study by developing procedures that identify, evaluate and control risk for all aspects of the study, e.g. study design, source data management, study team training, participant eligibility, and informed consent.

The Study Coordinating Centre will also implement quality control (QC) procedures, which will include the checks within the data entry system; any missing data or data anomalies will be communicated to the site(s) for clarification/resolution. QC activities will also be undertaken by study monitors, who will check that the study is conducted, and that data are generated, and biological specimens are collected, documented (recorded), and reported in compliance with the protocol, good clinical practice and applicable regulatory requirement at least annually.

In addition, each site will perform internal QC activities to check that study conduct, data and biological specimen collection, and essential documentation is in compliance with the protocol, good clinical practice and applicable regulatory requirements. An individualised quality management plan will be developed to describe a site's quality management.

In the event of non-compliance that significantly affects human participant protection or reliability of results, the Site PI and/or Study PI will perform a root cause analysis and corrective and preventative action plan (CAPA).

To standardise the collection of data by local Site Investigators and research coordinators, training of the Site Investigator and research coordinator will be undertaken by the Study Coordinating Centre team. This will include staff familiarisation of the protocol and procedures, provision of study-specific documentation, SOPs and manuals, attendance at site initiation visits (SIVs) and training on the BLUEPRINT web-based DMS, i.e. REDcap. The Site Investigator and research coordinator will train the remaining local clinical site team, in particular the medical/nursing staff conducting consent, enrolment and measurements. Training of the Site PI and research coordinator/study team will include all aspects of study conduct.

All sites will have access to the BLUEPRINT standard operating procedures contained within the Manual of Procedures document.

10.3 Study Monitoring

Study monitoring is undertaken to ensure that the rights and well-being of study infants are protected, that the reported study data are accurate, complete, and verifiable, and that the conduct of the study is in compliance with the currently approved protocol, with International Conference on Harmonisation Good Clinical Practice (ICH GCP), and with applicable regulatory requirement(s).

The Sponsor's monitoring frequency has been determined by an initial risk assessment performed prior to the start of the study. A detailed Clinical Monitoring Plan (CMP) has been developed detailing the frequency and scope of the monitoring for the study. Throughout the conduct of the study, the risk assessment will be reviewed, and the monitoring frequency adjusted, as necessary.

Remote and central monitoring will be conducted for all participating sites. The scope and frequency of monitoring has been determined by the risk assessment and detailed in the Clinical Monitoring Plan (CMP) for the study. Unresolved or significant findings from remote monitoring visits may result in the escalation from remote visits to on-site visits.

- Regular remote and central monitoring will be performed according to the study specific Clinical Monitoring Plan (CMP).
- Central monitoring will occur regularly focusing on targeted source data verification of scheduled study assessments.
- Remote monitoring will occur every 6 months focusing on site compliance with ICH-GCP, informed consent and recruitment processes and essential document management.
- Data will be evaluated for compliance with the protocol requirements and accuracy in relation to source documents as defined in the CMP.
- Following written standard operating procedures, the monitors will verify that the clinical study is conducted, and data are generated, documented, and reported in compliance with the protocol, GCP and the applicable regulatory requirements.

- Each participating site will be provided with copies of monitoring reports within 7 days of each remote monitoring visit.
- Independent audits may be conducted by the Sponsor or a representative of the Sponsor, to ensure monitoring practices are performed consistently across all participating sites and that monitors are following the CMP.

11 STATISTICAL METHODS

11.1 Sample Size Estimation

The sample size for BLUEPRINT is designed to address the robustness of early respiratory phenotypes identified predicting BPD. The prevalence of survival with BPD within the ANZNN is 0.69. Based on the R package pmsampsize version 1.1.3 described by Riley et al in *"Calculating the sample size required for developing a clinical prediction model"*,(36) we estimated that the following sample size will be required to develop a clinical risk prediction model with BPD (yes/no) as the outcome: assuming a BPD prevalence of 0.69 (69%; all grades) and a moderate C-index of 0.75 is achievable (latest estimates from PRELIM manuscript support this) with a binary logistic regression prediction model with BPD (yes/no) as the outcome, a minimum sample size of 500, 550 and 600 infants was estimated based on 10, 11 or 12 predictor variables respectively (from any domain of clinical, imaging and protein data). We estimate that the predictive model developed in BLUEPRINT may have up to 10-12 predictor variables which is partly based on the latest PRELIM manuscript where several basic clinical covariates (e.g. gender, gestational age) and only single proteins were included (given the limited sample size in PRELIM), however there were multiple proteins that were significantly correlated with BPD and there will be additional clinical covariates and other model terms (interactions, non-linear terms, which are counted as additional predictor variables terms in the pmsampsize sample size estimation) that will likely be critical to include in the BLUEPRINT study in order to achieve high performing predictive models.

The calculation above derived from the pmsampsize R package considers multiple criteria to arrive at an appropriate sample size that ensures precise predictions, minimises prediction errors and model overfitting/optimism, and allows for sufficient stratification by factors known to exert a strong signal on neonatal outcomes (e.g. gestational age/weight, sex and intubation in first 10 days).

This sample size prediction assumes the distribution of births within the 22-25+6-, 26-28+6- and 29-31+6-week GA cohorts as reported in the ANZNN. Between 2018-2020, approximately 750 infants aged 22-32 weeks' gestation were born per annum in Victoria who would have met the projects eligibility criteria; 37% <28 weeks' GA, overall mortality 7% (Data Safer Care Victoria). To achieve the desired sample size a recruitment rate of 20-25% of all eligible births (approximately 2250 births) would be required. Assuming a 5% loss to follow up/withdrawal rate between the first week after birth and BPD assessment a sample size of 550 infants included into the primary aim of the study (early life respiratory phenotype description) would allow 525 infants for inclusion in the BPD prediction modelling data set. This should also allow for approximately 400-450 surviving infants available for 2-year follow up (assuming >80% retainment rate after discharge).

11.2 Statistical Analysis Plan and Methods of Analysis

A separate Statistical Analysis Plan (SAP) will be developed by the Study Statistician(s) and SSC.

Defining the early life respiratory phenotypes: Proteome, lung imaging, clinical and demographic data will be combined to examine longitudinal alterations with ANOVA (multi-comparison), generalised additive models and post-hoc analysis using Perseus/R. Both supervised and unsupervised multivariate approaches including principal component analysis (PCA), Latent Class Analysis, hierarchical cluster analysis and partial least square discriminant analysis (PLS-DA) maybe used to assess i) which clinical/protein/imaging measures tend to cluster at each time-point, ii) how background changes in protein densities, functional imaging and cardiorespiratory measures relate to different clinical treatments. Significant correlations between candidate proteins, imaging, clinical and physiological markers will be identified with permutation tests with custom R scripts written by the Study Statistician(s) and multivariate visualisations (network maps) of important clinical-imaging-protein trait interactions (assessed with canonical correlation analysis) visualised. Phenotype discovery will target two broad sub-types: 1) known and novel disease-mechanisms and 2) outcome-based signatures.

Development of a biomarker-based BPD risk (and 1 and 2 year) prediction model: We will develop a risk prediction model for BPD diagnosed at 36 weeks, based on a combination of proteins, imaging and clinical measures (minimum 7 inputs) derived within the first 7 days after birth. Model development will include several steps starting with methods for variable selection and model fitting to testing the performance and validation of models. To test measures originally significantly associated with BPD, and identify other measures that are predictive of BPD, binary logistic regression, multiple forward stepwise logistic regression, and other classification models will be evaluated with likely measures as covariates. This approach is similar to other recent studies that developed prognostic, biomarker-based models for predicting adverse outcomes in adults with respiratory problems. (34) Model performance and discrimination will then be evaluated. Internal validation with both cross-validation and bootstrapping methods to adjust for model over-optimism and bias towards inherent overestimated performance metrics will be applied. A similar strategy will be used for developing and testing risk prediction models at 1 and 2 years, which will include initial variable selection and subsequent model performance (AUC, discrimination) and validation (cross-validation) tests. We will examine several methods (e.g. growth curve trajectory) for coding repeatedly measured predictor variables that best capture developmental changes related to lung injury over time and subsequent respiratory risks. Cox proportional-hazards regression models and Kaplan-Meier survival curves will be used to further investigate and illustrate differences in BPD risk outcomes between high-risk and low-risk groups based on prognostic scores derived from BPD risk models, which will be useful to account for infants that may be lost to follow-up before 2 years.

Testing and developing alternative machine learning models of BPD risk.

While binary logistic regression is commonly used for developing risk prediction models when disease outcomes are binary yes/no, it will be important to test alternative risk prediction models that may be better able to handle high dimensional data (protein data, imaging data) and capture nonlinear relationships between variables. Some studies have shown a slight improvement in model prediction performance between logistic regression (LR) and machine learning (ML) algorithms,(37) while others have not.(38) AI methods are showing particular promise for providing advancements in areas where disease prediction is based on complex data such as lung imaging(39) or protein data.(40) Given BLUEPRINT will test whether integration of traditional BPD risk factors with protein, imaging and other data may improve prediction of BPD outcomes, it will be important to test alternative risk prediction models. We will test several commonly used ML algorithms (e.g. K-nearest neighbour, random forests, support vector machine). LR and ML models will all be internally validated and compared using the same data. Model performance will be compared with accuracy, sensitivity and specificity.

11.3 Handling of missing data

Every attempt will be made to include complete data for all time points. This is not always possible with bedside ICU measurements or monitoring tools. It is possible that complete datasets of lung imaging, plasma samples and respiratory support data will not be possible. The SAP will detail the minimum data set for inclusion in analysis and how missing data will be handled. This will include rules regarding sources of missing data that can be imputed if there is partial information that can be used as a reference to impute the missing data. Variable imputation will include standard methods (e.g. polytomous regression for unordered categorical variables, Bayesian regression for continuous variables, LR for binary variables). Multiple imputation (e.g. with the R MICE package) may also be considered if the missingness mechanism (tested with Little's MCAR Test, correlation analysis) is not completely at random.

12 ETHICS AND DISSEMINATION

12.1 Research Ethics Approval & Local Governance Authorisation

This protocol and the informed consent document and any subsequent amendments will be reviewed and approved by the human research ethics committee (HREC) prior to commencing the research. A letter of protocol approval by HREC will be obtained prior to the commencement of the study, as well as approval for other study documents requiring HREC review.

Each participating institution will also obtain institutional governance authorisation for the research and associated HREC-approved documents. A letter of authorisation will be obtained from the RGO prior to the commencement of the research at that institution. Institutional governance authorisation for any subsequent HREC-approved amendments will be obtained prior to implementation at each site.

12.2 Amendments to the protocol

This study will be conducted in compliance with the current version of the protocol. Any change to the protocol document or Informed Consent Form that affects the scientific intent, study design, participant safety, or may affect a participant's willingness to continue participation in the study is considered an amendment, and therefore will be written and filed as an amendment to this protocol and/or informed consent form. All such amendments will be submitted to the HREC, for approval prior to being implemented.

12.3 Protocol deviations and serious breaches

All protocol deviations will be recorded in the participant record (source document) and on the CRF in the BLUEPRINT Study data management system (DMS) and must be reported to the Site PI and Coordinating Principal Investigator, who will assess for significance. Those deviations deemed to affect the rights of a study participant, or the reliability and robustness of the data generated in the clinical study to a significant degree will be reported as serious breaches. Reporting will be done in a timely manner:

- The Site PI will report to the CPI within 72 hours and to the Site RGO within 7 days; the CPI will review and submit to the approving HREC within 7 days.

Where non-compliance significantly affects human participant protection or reliability of results, a root cause analysis will be undertaken, and a corrective and preventative action plan prepared.

Where protocol deviations or serious breaches identify protocol-related issues, the protocol will be reviewed and, where indicated, amended.

Any failure to follow a major component of the protocol will result in a protocol deviation. Examples of a protocol deviations include:

- Failure to follow data collection as per the SoA.
- Enrolment of an infant who is ineligible for the study.
- Failure to follow the approved study protocol that affects participant safety or data integrity.
- Failure to report serious adverse event to the Sponsor/TCC.
- Continuing research activities after HREC approval has expired.

The BLUEPRINT Manual of Procedures details procedures for reporting protocol deviations to the Sponsor.

13 PARTICIPANT REIMBURSEMENT

Car parking vouchers (or equivalent public transport reimbursement) and food vouchers for the Melbourne Children's Campus precinct to a maximum of $30/visit will be provided to the parent/legal guardian of each participating infant to attend the 1- and 2- year follow up assessments at the MCRI. There will be no other participant reimbursement for the BLUEPRINT Study.

14 FINANCIAL DISCLOSURE AND CONFLICTS OF INTEREST

Prue Pereira-Fantini, Sean Byars, David Tingay, Peter Davis and Brett Manley hold provision patent (2023902862) filed with IP ustralia related to protein biomarkers of preterm lung injury.

There are no other financial or competing interests for Investigators for the overall study and each study site.

15 DISSEMINATION AND TRANSLATION PLAN

The Study Coordinating Centre and/or Data Coordinating Centre will distribute study information to participating sites via the following tools:

1. BLUEPRINT study website
2. Distribution lists/email correspondence
3. Follow up reminder support (e.g. fridge magnets)
4. BLUEPRINT study REDcap DMS help desk at the MCRI; and
5. Direct access to the STUDY COORDINATING CENTRE members.

In addition, a BLUEPRINT study newsletter for participating sites will be disseminated on a regular basis. At study completion, a summary of the study results will also be distributed to participating families.

15.1 Publication Policy

All BLUEPRINT study manuscripts and abstracts ("publications") must, before submission, be reviewed by the BLUEPRINT SSC. The Steering Committee will form the writing committee of the main primary paper. The author line must conclude with "and the BLUEPRINT study Investigators," and NHMRC (and any additional) funding must be acknowledged, specifying the grant number if applicable.

Note: "publications" include abstracts and posters for presentation at national and international meetings.

Manuscripts are assigned a primary reviewer(s) from the SSC who is responsible for final approval. The manuscript is returned to the lead author with major comments (required changes) and minor comments (recommended changes). If there are required changes, the manuscript must be revised and resubmitted for further review. This process is repeated until no required changes remain. In case of persistent disagreement between authors and reviewers, final judgment rests with the STUDY COORDINATING CENTRE chair. Abstracts undergo a similar but abbreviated review.

Additionally, if the analysis is done at the local site: the lead author is required to submit the analytical plan and computer code used to produce the results in the publication from the original dataset. If results cannot be reproduced, the publication will not be approved for submission.

No ancillary study can be published until the primary paper(s) are published.

15.2 Authorship

BLUEPRINT supports and subscribes to the policies of the International Committee of Medical Journal Editors' Uniform Requirements for Manuscripts Submitted to Biomedical Journals (http://www.icmje.org/). These Requirements state:

"Authorship credit should be based on:

1) Substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data;

2) Drafting the article or revising it critically for important intellectual content; and

3) Final approval of the version to be published.

Authors should meet conditions 1, 2, and 3."

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Where journal policies permit, all Investigators who played a contributing role in the study, including to its accrual, will be included in an Acknowledgement section. DCCe and site staff with direct project-specific responsibilities will also be acknowledged.

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