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Autologous transplantation of umbilical cord blood derived stem cells in extreme preterm infants: protocol for a safety and feasibility study

CORD-SAFE Study

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ABSTRACT

Introduction: Preterm brain injury continues to be an important complication of preterm birth, especially in extremely premature infants. Umbilical cord blood derived cells (UCBCs) are being increasingly evaluated for their neuroprotective and neuroreparative properties in preclinical and clinical studies. There remains a paucity of information on the feasibility and safety of autologous UCBC transplantation in extremely premature infants.

Methods and Analysis: A single centre safety and feasibility study in preterm babies born before 28 weeks gestation. Cord blood will be collected after birth and, if sufficient blood is obtained, UCBC mononuclear fractions will be harvested from the cord blood, characterised and stored. After excluding infants who have already suffered severe preterm brain injury, based on cranial ultrasound in first week of life, preterm infants will be infused with autologous UCBC via the intravenous route at a dose of between 25-50 million UCBCs/kg body weight of live cells, with the cell number being the maximum available up to 50 million cells. A minimum of 20 infants will be administered autologous UCBCs. Primary outcomes will include feasibility and safety. Feasibility will be determined as access to sufficient cord blood at collection and UCBCs following processing. Safety will be determined by lack of adverse events directly related to autologous UCBC administration in the first few days after cell administration. Secondary outcomes studied will include neonatal, and neurodevelopmental morbidities till 2 years of life. Additional outcomes will include cord blood cell characterisation of all enrolled infants, and cytokine responses to cell administration in transplanted infants till 36 weeks corrected age.

Ethics and Dissemination: Monash Health Human Research Ethics Committee approved this study in December 2019. Recruitment is to commence in January 2020 and is expected to take around 12 months. The findings of this study will be disseminated via peer-reviewed journals and at conferences.

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Strengths and limitations of the study

- A world first study to evaluate the safety and feasibility of autologous umbilical cord blood collection and cell infusion in extreme premature infants.
- A study translating preclinical advances in cell based therapies for perinatal brain injury to the clinical arena.
- Assessment of preterm cord blood cell characteristics and cytokine profiling of response to cell transplantation that may inform mechanisms of action of UCBCs and the design of future efficacy trials.
- Given the phase 1 nature of the study, there are no prospective controls or randomisation.

INTRODUCTION

More than 3000 very preterm infants (those born less than 32 weeks' gestation) are born every year in Australia and around 7% of them go on to develop cerebral palsy (CP). Preterm brain injury (especially severe intraventricular haemorrhage and cystic periventricular leukomalacia) complicates the neonatal course of very preterm infants (especially extremely preterm infants; those born before 28 weeks' gestation), contributing as the principal cause of CP in this population (1). Most preterm infants who suffer severe or significant preterm brain injury in the neonatal period have very poor prognosis, with a proportion of them dying during the neonatal period, needing shunt surgery for post haemorrhagic hydrocephalus and, in those who survive, many go on to suffer from adverse long-term neurodevelopment outcomes, including CP. Advances in neonatal care have substantially improved survival in very preterm and extremely preterm infants with brain injury, but no early intervention curative treatment is currently available for preterm brain injury.

Cell therapies are increasingly being tested for neuroprotection and neuroregeneration in young children (2-5), including one current trial using sibling UCB in young children with confirmed CP in Australia (ACTRN12616000403437). Further trials are proposed. There is now substantial preclinical evidence that administration of umbilical cord blood derived mononuclear cells (UCBCs) in the early neonatal period reduces perinatal brain injury and prevents the progression of neuropathology and CP in the long term. UCBCs have been shown to be neuroprotective for the preterm brain when administered in pre-clinical models of hypoxic-ischemic and inflammation-induced preterm brain injury (6-8). More recently, we have shown that a single dose of UCBC therapy delivered improvement in long-term behavioural outcomes in a rat model of neonatal hypoxic ischemic injury (9). Umbilical cord blood can be easily and safely collected at birth, leading to a high cell yield which contains a wide variety of stem and progenitor cells that have been shown to mediate positive benefits on a variety of neurological cells, including glial cells, neurons and cells that maintain the

blood brain barrier (BBB) (2, 10-12). These, and other, studies show that the neuroprotective and neuroreparative benefits of early UCBC therapy for the developing brain are likely mediated by their anti-apoptotic, anti-inflammatory, pro-angiogenic, neurogenic, antioxidant, and BBB protective mechanisms (4, 6-8). Most of these studies have used term cord blood-derived UCBCs, but we have also investigated the neuroprotective/neuroreparative characteristics of preterm UCBCs (6, 13). There are only two published clinical studies, which have been conducted, in preterm infants using autologous UCBCs, but the gestation age of included infants was greater than 28 weeks (14, 15).

We hypothesise that in extremely premature infants, i) autologous umbilical cord blood cell (UCBC) collection at birth will be feasible and the ii) intravenous administration of umbilical cord blood derived stem cells in the neonatal period will be safe. The specific aims of this study, thus, are to test the feasibility of collection of sufficient UCBC from cord blood; and safety of autologous intravenous UCBC administration in extremely preterm infants during the neonatal period.

METHODS AND ANALYSIS

Design: Phase 1, single centre feasibility and safety study.

Setting: Monash Newborn, Monash Children's Hospital, Monash Health.

Participants: Extreme preterm infants born before 28 completed weeks of gestation (up to 27^{+6} weeks).

Sample size: We aim to administer UCBCs to at least 20 extremely preterm infants at the dose range detailed below. The sample size is consistent with other phase 1 UCBC trials conducted in newborn infants (2, 14). The number of recruited infants and cord blood collections will exceed 20 as we may not have enough cord blood collected after birth, or

may not have enough UCBCs available for administration after processing of the cord blood. Further, some infants may be excluded after cord blood collection as per exclusion criteria below. See participant flow in Figure 1.

Cord blood collection

Cord blood collection and processing: Umbilical cord blood will be collected from all eligible extreme preterm infants of parents who have consented. Size appropriate needle and collection containers/ bags will be used to maximise the volume of cord blood that can be obtained. Minimum cord blood collection volume for inclusion in the trial will be 9 mLs. Infants with collected cord volume of less than 9 mLs will be excluded from the trial, but their cord blood will be retained and its characteristics investigated as part of a sub-study. The collected cord blood (≥9 mLs) will be processed according to standard cord blood processing operating procedures, aliquoted and cryopreserved.

Storage and release: An aliquot containing sufficient numbers of UCBCs, according to weight of the preterm infant, required for early administration (see dose below) will be stored. Any excess UCBCs will compose further aliquot(s) that will be stored separately for potential subsequent use, not comprising part of the current trial. Cell Care (Heatherton, VIC), a TGA accredited cord blood bank, will be responsible for collection, processing, storage of all samples and release of UCBC for autologous administration to the preterm infants. Standard testing for impurities, cell viability and DNA matching of UCBC to the preterm infant's DNA will be performed before release of UCBCs. Criteria for product release will include: free of microbial contamination after 7 days of culture, cell viability > 70% as determined by trypan blue exclusion at the time of cryopreservation and following dilution prior to administration, after thawing and washing of cell and DNA match of cord blood and infant sample.

Thawing and infusion preparation: On the day of infusion, UCBCs will be retrieved from liquid nitrogen storage, and, once product release criteria are met, the frozen UCBC aliquot

will be transported to Monash Health. Cells will be thawed using a pre-warmed bead bath for approximately 2 minutes. The UCBCs will be washed with dextrose/ albumin and centrifuged at 350g for 5 minutes prior to resuspension in dextrose/ albumin, viability testing and counting of live cell number and dilution to the final desired concentration. The UCBCs will be suspended at a concentration 10% greater than the desired concentration to allow for cell loss. Volume of cell infusion prepared will be around 10 mL/kg body weight.

UCBC administration

Inclusion criteria: Extreme preterm infants with absence of severe brain injury on neonatal cranial ultrasound, performed in the first week of life.

Exclusion criteria: Extreme preterm infants with evidence of severe preterm brain injury as defined by Grade III-IV intraventricular haemorrhage (16) and/ or cystic periventricular leukomalacia. Infants who are likely to have redirection of intensive care due to any reason, as decided by the treating clinical team would also be excluded.

Intervention: UCBCs from autologous cord blood will be administered intravenously to infants, between D9 – D15 of life. This will follow a routine D8 cranial ultrasound scan. Infants with an active bacterial infection (blood culture positive in last 48 hours) or instability as determined by the treating team will have cell administration deferred, for subsequent administration, if possible, within the treatment window (D9-15).

Dose: UCBCs will be administered intravenously (through a peripheral intravenous catheter) at a dose of 25-50 million viable cells/ kg body weight, 25 million/kg being the proposed minimum dose, and 50 million/kg being the maximum dose. The dose will depend on the number of UCBCs able to be processed and deemed viable after cord blood collection. This dose is based on preclinical (6, 7, 13, 17) and clinical studies (2, 14) on UCBC use for perinatal brain injury. If the number of cells available is less than 25 million/kg, then cell

administration will not occur in this trial but cell characteristics will be studied. Volume of cell infusion will be around 10 mL/kg and cell infusion will occur over 30 minutes. The cell administration protocol will be based on an ongoing current placental stem cell study at Monash Newborn (18).

Cell infusion protocol: Infants will receive an infusion of UCBCs via a peripheral venous catheter. The suspension with UCBCs will be filtered through an inline IV filter. The infusion will be administered over 1 hour via a vertical syringe pump. No other medications, blood products or solutions will be infused via the same peripheral intravenous site during the UCBC infusion.

Primary outcomes: The primary aims of this study are to test i) feasibility of autologous cord blood collection and cell retrieval following processing from extremely premature infants; and ii) safety of autologous UCBC administration in eligible extremely preterm infants.

Feasibility will be determined by agreement to participate, ability to collect and process sufficient cord blood, and then access to sufficient UCBCs within the second week of life to meet the infusion criteria. UCBCs will be collected from all potentially eligible infants but will only be administered to eligible infants as per inclusion and exclusion criteria above.

Safety of UCBC administration will be determined by occurrence of adverse events as defined below. Infants will be monitored as follows.

Monitoring during UCBC infusion and nursery stay

Infants will be observed for 2 hours prior to UCBC infusion to determine their baseline cardiorespiratory status and establish acceptable parameters for fluctuations during the infusion. During the infusion, HR, RR, ECG, SpO_2 will be monitored continuously while BP, temperature, and site of infusion will be checked every 15 minutes. Post infusion, HR, RR,

ECG, SpO2 will be continuously monitored, while BP, and temperature will be checked hourly.

Infants, as a result of their prematurity, are likely to remain inpatients in intensive and special care nurseries for at least 3-4 months following the UCBC administration. The routine clinical care afforded infants over this time will serve as monitoring for adverse events.
Routine care will include continuous cardiorespiratory monitoring; physical examination (daily while infants remains on respiratory support); anthropometry (weekly weight, head circumference, length); documentation of respiratory support requirements; chest radiograph, as clinically indicated; blood gas analysis, as determined by clinical team; cranial ultrasound, as per clinical practice but a minimum of two cranial ultrasounds post infusion prior to discharge (D28, 42); term equivalent age MRI brain, as per clinical practice.

Monitoring post discharge

Following discharge from nursery, infants will be assessed at 6, 12, 18 and 24 months corrected age. Assessment will focus on general health including growth parameters and physical examination, reporting of any adverse events and medication usage. Neurodevelopmental assessment will be performed using standardised assessment tools, including General Movements and Hammersmith Infant Neurological Examination (at 3 months corrected age), and the Bayley Scales of Infants and Toddler Development (IV edition) at 2 years corrected age.

Defining Adverse Events

Adverse events will be defined as follows:

- During infusion
    - Local Site Reaction (Erythema, oedema, extravasation at site of peripheral intravenous catheter site)

- Any sustained change of 30% or more from baseline in vital signs (HR, RR, BP, SpO2, Temp)

- Within first 24 hours of infusion
  - Any event requiring cardiopulmonary resuscitation
  - Escalation of respiratory support (intubation of an infant receiving non-invasive respiratory support; or change to high frequency oscillatory ventilation in an infant receiving conventional ventilation at the time of UCBC infusion)
  - Fluid bolus or initiation/escalation of inotropic support
- Infection within 48 hours of UCBC infusion (culture proven bacterial, fungal or viral infection, or culture negative, clinically suspected infection)

Data Safety Monitoring Board (DSMB): An independent DSMB has been formed comprising a neonatologist, and a cell scientist/ biologist to review all adverse incidents. Adverse incidents will be reported to DSMB within 1 week of occurrence.

Reporting to HREC: The following reports will be submitted to HREC:

1. Any adverse event/ reaction deemed suitable by DSMB to temporarily halt the trial pending review.
2. An interim report after UCBC administration to 10 babies.
3. Annual research progress report.
4. Any updates to protocol/ PICF.

Secondary outcomes: Neonatal and long term general health and neurological outcomes, including cranial ultrasound changes after D8 of life, term equivalent MRI brain findings (if clinically indicated), early neurodevelopment assessments in the first three months of corrected age (General Movements, Hammersmith neonatal/ infant neurological examinations), and 2 year neurodevelopment assessment (BSID-IV). The secondary

outcomes of the study participants may also be compared to a matched historical cohort (same criteria of no evidence of severe brain injury by D8 scan) of extremely premature infants.

In a sub study, we will characterise the composition, characteristics and functionality of the preterm UCBCs collected from all enrolled infants. Comparative studies with stored term UCBCs collected for other studies will be considered.

We will also conduct a targeted cytokine analysis on infant's serum; namely IL-1b, IL-6, TNF-α and IL-10 at time (just before) of UCBC administration, 1 day post administration and at 36 -37 weeks postconceptional age as potential biomarkers for response to cell therapy. These cytokines have been shown to be impacted by UCBC administration in previous studies (7, 8).

Statistical Methods: As this is a phase I trial, detailed statistical analysis will not be required, but descriptive and inferential statistical analysis will be conducted as appropriate. Comparative analysis will be performed between study participants and historical controls.

ETHICS AND DISSEMINATION

Ethics: The study will be conducted in compliance with the approved protocol/amendment(s), conditions of Monash Health HREC approval and the NHMRC National Statement on Ethical conduct in Human Research 2007 (updated May 2018). A patient consumer representative was involved in the ethical preparation and review of this protocol.

Consent: Fully informed and written consent will be obtained antenatally from the parents after detailed discussion regarding the 'experimental' nature of this study, and potential lack of benefits of cord blood collection and/ or UCBC administration. Consent will not be obtained during active labour or after birth. In all cases, written consent will be obtained

using a specifically designed Participant Information and Consent Form. Participation will be voluntary and withdrawal, possible at any stage. Should withdrawal occur after an infant receives the intervention, safety monitoring will be offered in line with the monitoring outlined in the protocol.

The outcomes of this study will be disseminated via peer-reviewed journals and presented at scientific conferences. A summary report will be made available to all participant families.

AUTHOR CONTRIBUTIONS

All authors contributed to the design of the study. AM drafted the initial protocol and manuscript. IN, SLM and GJ made critical revisions and edited the manuscript. All authors contributed to and approved the final manuscript.

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Cell collection, processing, storage and release will be conducted by Cell Care Australia. Cell Care was not involved in the design of the trial.

COMPETING INTERESTS STATEMENT

The authors have no competing interests.

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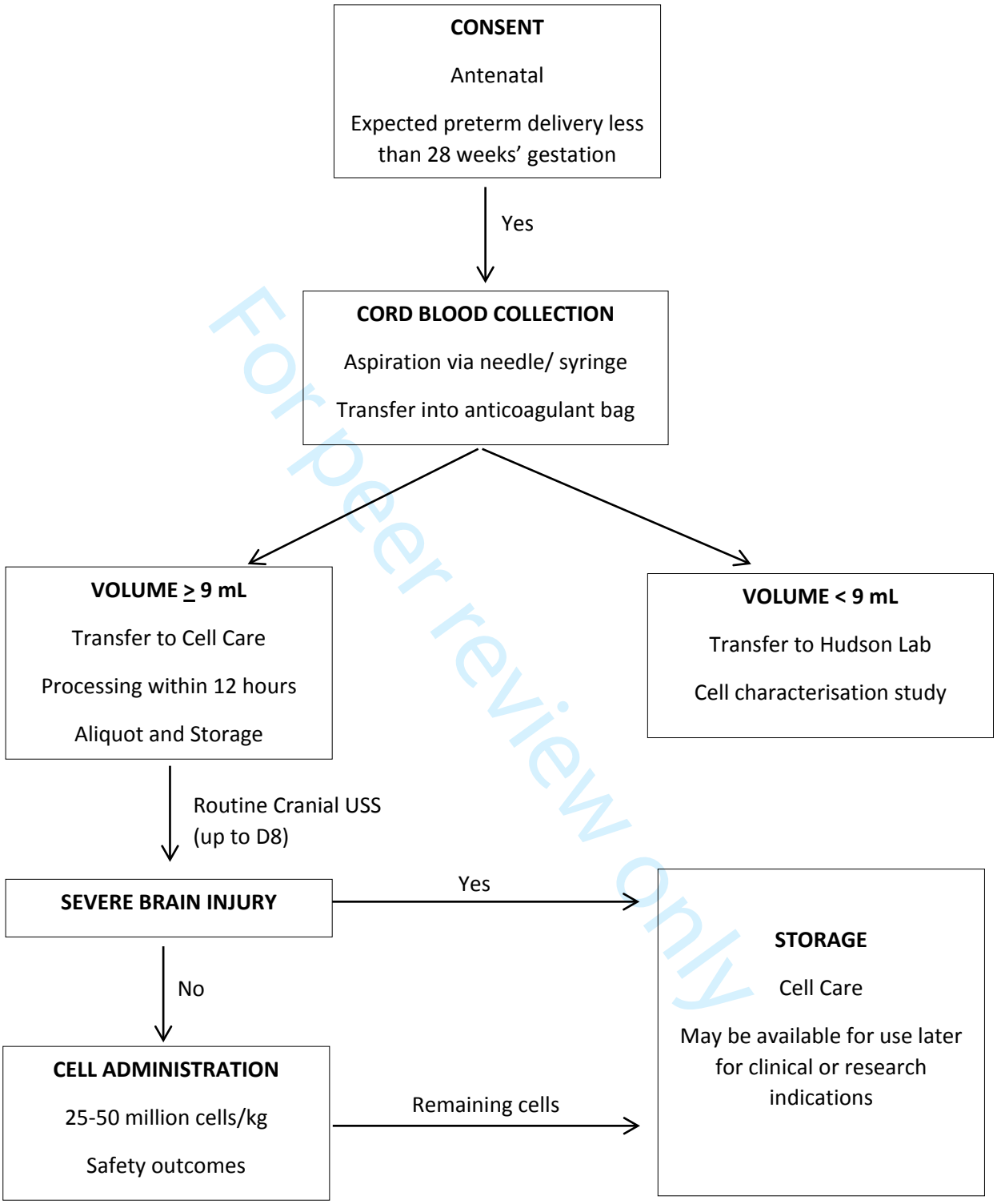
Figure 1. Participant flow



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Setting: Monash Newborn, Monash Children's Hospital, Monash Health.

Participants: Extreme preterm infants born before 28 completed weeks of gestation (up to 27^{+6} weeks).

Sample size: We aim to administer UCBCs to at least 20 extremely preterm infants at the dose range detailed below. The sample size is consistent with other phase 1 UCBC trials

conducted in newborn infants (3, 16). The number of recruited infants and cord blood collections will exceed 20 as we may not have enough cord blood collected after birth, or may not have enough UCBCs available for administration after processing of the cord blood. Further, some infants may be excluded after cord blood collection as per exclusion criteria below. See participant flow in Figure 1.

Cord blood collection

Cord blood collection and processing: Umbilical cord blood will be collected from all eligible extreme preterm infants of parents who have consented. Size appropriate needle (18-21 G) and collection containers/ bags (Macopharma, Tourcoing, France) will be used to maximise the volume of cord blood that can be obtained. Minimum cord blood collection volume for inclusion in the trial will be 9 mLs. Infants with collected cord blood volume of less than 9 mLs will be excluded from the trial, but their cord blood will be retained and its characteristics investigated as part of a sub-study. For all other infants, the collected cord blood (≥9 mLs) will be processed according to standard cord blood processing procedures, aliquoted and cryopreserved (18, 19).

Storage and release: An aliquot containing sufficient numbers of UCBCs, according to weight of the preterm infant, required for early administration (see dose below) will be stored. Any excess UCBCs will compose further aliquot(s) that will be stored separately for potential subsequent use, not comprising part of the current trial. Cell Care (Heatherton, VIC), a TGA accredited cord blood bank, will be responsible for collection, processing, storage of all cord blood and release of UCBCs for autologous administration to the preterm infants. Standard testing for microbes, cell viability and DNA matching of UCBC to the preterm infant's DNA will be performed before release of UCBCs. Criteria for product release will include: free of microbial contamination after 7 days of culture, cell viability > 70% as determined by trypan blue exclusion at the time of cryopreservation and following prior to administration, after thawing and washing of cryopreserved cells..

Thawing and infusion preparation: On the day of infusion, UCBCs will be retrieved from liquid nitrogen storage, and, once product release criteria are met, the frozen UCBC aliquot will be transported to Monash Health. Cells will be thawed using a pre-warmed bead bath for approximately 2 minutes. The UCBCs will be washed with dextrose/ albumin and centrifuged at 350g for 5 minutes prior to resuspension in dextrose/ albumin, viability testing and counting of live cell number and dilution to the final desired concentration. The UCBCs will be suspended at a concentration 10% greater than the desired concentration to allow for cell loss. Volume of cell infusion prepared will be around 10 mL/kg body weight.

UCBC administration

Inclusion criteria: Extreme preterm infants with absence of severe brain injury on neonatal cranial ultrasound, performed in the first week of life.

Exclusion criteria: Extreme preterm infants with evidence of severe preterm brain injury as defined by Grade III-IV intraventricular haemorrhage (20) and/ or cystic periventricular leukomalacia. Infants who are likely to have redirection of intensive care due to any reason, as decided by the treating clinical team would also be excluded.

Intervention: UCBCs from autologous cord blood will be administered intravenously to infants, between D9 – D15 of life. This will follow a routine D8 cranial ultrasound scan. Infants with an active bacterial infection (blood culture positive in last 48 hours) or instability as determined by the treating team will have cell administration deferred, for administration, if possible, within the treatment window (D9-15).

Dose: UCBCs will be administered intravenously (through a peripheral intravenous catheter) at a dose of 25-50 million viable cells/ kg body weight, 25 million/kg being the proposed minimum dose, and 50 million/kg being the maximum dose. The dose will depend on the

number of UCBCs able to be processed and deemed viable after cord blood collection. This dose is based on preclinical (7, 8, 10, 15, 21) and clinical studies (3, 16) on UCBC use for perinatal brain injury. If the number of cells available is less than 25 million/kg, then cell administration will not occur in this trial but cell characteristics will be studied. These extreme premature infants frequently receive blood products (packed red blood cells, plasma, and rarely platelets at volumes of 10-15 mL/kg, and hence it was decided that the volume of cell infusion in this study will also be around 10 mL/kg. The cell administration protocol will be based on an ongoing current placental stem cell study at Monash Newborn (22).

Cell infusion protocol: Infants will receive an infusion of UCBCs via a peripheral venous catheter. The suspension with UCBCs will be filtered through an inline IV filter. The infusion will be administered over 30 minutes via a vertical syringe pump. No other medications, blood products or solutions will be infused via the same peripheral intravenous site during the UCBC infusion.

Primary outcomes: The primary aims of this study are to test i) feasibility of autologous cord blood collection and cell retrieval following processing from extremely premature infants; and ii) safety of autologous UCBC administration in eligible extremely preterm infants.

Feasibility will be determined by agreement to participate, ability to collect and process sufficient cord blood, and then access to sufficient UCBCs within the second week of life to meet the infusion criteria. UCBCs will be collected from all potentially eligible infants but will only be administered to eligible infants as per inclusion and exclusion criteria above.

Safety of UCBC administration will be determined by occurrence of adverse events as defined below. Infants will be monitored as follows.

Monitoring during UCBC infusion and nursery stay

Infants will be observed for 2 hours prior to UCBC infusion to determine their baseline cardiorespiratory status and establish acceptable parameters for fluctuations during the infusion. During the infusion, HR, RR, ECG, SpO_2 will be monitored continuously while BP, temperature, and site of infusion will be checked every 15 minutes. Post infusion, HR, RR, ECG, SpO2 will be continuously monitored, while BP, and temperature will be checked hourly.

Infants, as a result of their prematurity, are likely to remain inpatients in intensive and special care nurseries for at least 3-4 months following the UCBC administration. The routine clinical care afforded infants over this time will serve as monitoring for adverse events.
Routine care will include continuous cardiorespiratory monitoring; physical examination (daily while infants remains on respiratory support); anthropometry (weekly weight, head circumference, length); documentation of respiratory support requirements; chest radiograph, as clinically indicated; blood gas analysis, as determined by clinical team; cranial ultrasound, as per clinical practice but a minimum of two cranial ultrasounds post infusion prior to discharge (D28, 42); term equivalent age MRI brain, as per clinical practice.

Monitoring post discharge

Following discharge from nursery, infants will be assessed at 6, 12, 18 and 24 months corrected age. Assessment will focus on general health including growth parameters and physical examination, reporting of any adverse events and medication usage. Neurodevelopmental assessment will be performed using standardised assessment tools, including General Movements and Hammersmith Infant Neurological Examination (at 3 months corrected age), and the Bayley Scales of Infants and Toddler Development (IV edition) at 2 years corrected age.

Defining Adverse Events

Adverse events will be defined as follows:

- During infusion
    - Local Site Reaction (Erythema, oedema, extravasation at site of peripheral intravenous catheter site)
    - Any sustained change of 30% or more from baseline in vital signs (HR, RR, BP, SpO2, Temp)
- Within first 24 hours of infusion
    - Any event requiring cardiopulmonary resuscitation
    - Escalation of respiratory support (intubation of an infant receiving non-invasive respiratory support; or change to high frequency oscillatory ventilation in an infant receiving conventional ventilation at the time of UCBC infusion)
    - Fluid bolus or initiation/escalation of inotropic support
- Infection within 48 hours of UCBC infusion (culture proven bacterial, fungal or viral infection, or culture negative, clinically suspected infection)

Data Safety Monitoring Board (DSMB): An independent DSMB has been formed comprising a neonatologist, and a cell scientist/ biologist to review all adverse incidents. Adverse incidents will be reported to DSMB within 1 week of occurrence.

Reporting to HREC: The following reports will be submitted to HREC:

1. Any adverse event/ reaction deemed suitable by DSMB to temporarily halt the trial pending review.
2. An interim report after UCBC administration to 10 babies.
3. Annual research progress report.
4. Any updates to protocol/ PICF.

Secondary outcomes: Neonatal and long term general health and neurological outcomes, including cranial ultrasound changes after D8 of life (although unlikely to impact IVH

outcomes as IVH is more a complication in the first week of life), term equivalent MRI brain findings (if clinically indicated), early neurodevelopment assessments in the first three months of corrected age (General Movements, Hammersmith neonatal/ infant neurological examinations), and 2 year neurodevelopment assessment (BSID-IV). The secondary outcomes of the study participants may also be compared to a matched historical cohort (same criteria of no evidence of severe brain injury by D8 scan) of extremely premature infants.

In a sub study, we will characterise the composition, characteristics and functionality of the preterm UCBCs collected from all enrolled infants. Comparative studies with stored term UCBCs collected for other studies will be considered.

We will also conduct a targeted cytokine analysis on infant's serum; namely IL-1b, IL-6, TNF-α and IL-10 at time (just before) of UCBC administration, 1 day post administration and at 36 -37 weeks postconceptional age as potential biomarkers for response to cell therapy. These cytokines have been shown to be impacted by UCBC administration in previous studies (8, 9).

Statistical Methods: As this is a phase I trial, detailed statistical analysis will not be required, but descriptive and inferential statistical analysis will be conducted as appropriate. Comparative analysis will be performed between study participants and historical matched controls.

ETHICS AND DISSEMINATION

Ethics: The study will be conducted in compliance with the approved protocol/amendment(s), conditions of Monash Health HREC approval and the NHMRC National Statement on Ethical conduct in Human Research 2007 (updated May 2018). Expert neonatal clinical review panel and hospital ethics committee deliberated with the research team to determine the

inclusion criteria. It was felt due to lack of acceptability by clinicians, and in the absence of safety evidence of UCBC transplantation in extremely premature infants; the trial should first include infants without severe brain injury to establish feasibility and safety. A patient consumer representative was involved in the ethics preparation and review of this protocol.

Consent: Fully informed and written consent will be obtained antenatally from the parents after detailed discussion regarding the 'experimental' nature of this study, and potential lack of benefits of cord blood collection and/ or UCBC administration. Consent will not be obtained during active labour or after birth. In all cases, written consent will be obtained using a specifically designed Participant Information and Consent Form. Participation will be voluntary and withdrawal, possible at any stage. Should withdrawal occur after an infant receives the intervention, safety monitoring will be offered in line with the monitoring outlined in the protocol.

Patient and public involvement: Consumer input and feedback through the hospital ethics committee and cerebral palsy alliance (a patient advocacy non-profit organisation) consumer groups was sought in the design of this and other cord blood trials. A summary report will be made available to all participant families.

The outcomes of this study will be disseminated via peer-reviewed journals and presented at scientific conferences.

AUTHOR CONTRIBUTIONS

All authors contributed to the design of the study. AM drafted the initial protocol and manuscript. IN, SLM and GJ made critical revisions and edited the manuscript. All authors contributed to and approved the final manuscript.

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Cell collection, processing, storage and release will be conducted by Cell Care Australia.

Cell Care was not involved in the design of the trial.

COMPETING INTERESTS STATEMENT

The authors have no competing interests.

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Figure legends

Figure 1. Participant flow