Study identifier:
Safety of sib CB for CP

Full study title:
Safety study of sibling cord blood cell infusion to children with cerebral palsy

Lay study title:
Stem Cells in Umbilical Blood Infusion for CP (SCUBI-CP)

HREC/14/RCHM/38

RCH HREC ID: 34210

Version 10, 6th March 2017

CONFIDENTIAL

STATEMENT OF COMPLIANCE

This document is a protocol for a clinical trial. The study will be conducted in compliance with all stipulations of this protocol, the conditions of ethics committee approval, the NHMRC National Statement on Ethical Conduct in Human Research (2007) and the Note for Guidance on Good Clinical Practice (CPMP/ICH-135/95).

CONTENTS

PROTOCOL SYNOPSIS

Title	Safety study of sibling cord blood cell infusion to children with cerebral palsy
Objectives	The primary objective of this study is to gain preliminary information on the safety of 12/12 HLA matched umbilical cord blood cell (UCBC) infusion in children with cerebral palsy (CP). The secondary objectives of this study are: A) to gain preliminary information on the treatment effect of 12/12 HLA matched UCBC infusion B) to better understand the length of time that infused matched sibling UCBCs remain within recipients C) to gather information and samples for future studies into mechanistic activity of UCBCs
Trial sites	The Royal Children's Hospital Melbourne Lady Cilento Children's Hospital Brisbane The Children's Hospital at Westmead (recruitment) Monash Health (recruitment)
Participants	Number to be enrolled for screening: 48 Number to continue through trial: 12; Age: 1 - 16 years old

GLOSSARY OF ABBREVIATIONS

ABBREVIATION	TERM
AusCord	Australian National Network of Umbilical Cord Blood Banks and Cord Blood Collection Centres
ABMDR	Australian Bone Marrow Donor Registry
ABMTRR	Australasian Bone Marrow Transplant Recipient Registry
AE	Adverse event
BMDI CBB	BMDI Cord Blood Bank
BMI	Body mass index
CBB	Cord blood bank
CBU	Cord blood unit
CHW	The Children's Hospital at Westmead
CIBMTR	Center for International Blood & Marrow Transplant Research
CP	Cerebral palsy
DMSO	Dimethyl sulfoxide
EPO	Erythropoietin
GMFCS	Gross motor function classification system
GvHD	Graft-versus-host disease
HLA	Human leucocyte antigen
LCCH	Lady Cilento Children's Hospital Brisbane
MCRI	Murdoch Childrens Research Institute
MNC	Mononuclear cells
MRI	Magnetic resonance imaging
NSW CPR	New South Wales Cerebral Palsy Register
OT	Occupational therapy
PT	Physiotherapy
PVC	Peripheral venous catheter
QCPR	Queensland Cerebral Palsy Register
RCH, Melbourne	The Royal Children's Hospital Melbourne
RCT	Randomised controlled trial
SAE	Serious adverse event
SCBB	Sydney Cord Blood Bank
SOP	Standard operating procedure
TNC	Total nucleated cells
TRECs	T-cell receptor excision circles
UCB	Umbilical cord blood
UCBCs	Umbilical cord blood cells
VCPR	Victorian Cerebral Palsy Register

1. INVESTIGATORS AND FACILITIES

1.1. Study locations

Infusion sites:

State	*Site*
Victoria	The Royal Children's Hospital Melbourne
Queensland	Lady Cilento Children's Hospital Brisbane*

* Optional

Other study procedures:

State	*Site*
Victoria	The Royal Children's Hospital Melbourne Monash Health
New South Wales	The Children's Hospital at Westmead
Queensland	Lady Cilento Children's Hospital Brisbane
All states	Locally as required

1.2. Study management

The study will be managed by a research team consisting of principal investigators, associate investigators and clinical trial coordinators who will manage the daily operations such as recruitment, follow up assessments and study documentation. This is a multisite clinical trial, and each site will be managed by a different Principal Investigator. Across the sites, there will be a number of teams for different aspects of the trial, each with a designated team leader (please see below).

This trial is investigator-initiated by a collaborative research group. This trial does not have a sponsor.

Principal Investigators

Principal Investigators at each site will coordinate the supporting departments within the hospital. They will undertake site meetings and have access to a clinical trial coordinator working in each state.

The Royal Children's Hospital Melbourne (test site, lead HREC)

Prof Dinah Reddihough is group leader of Developmental Disability and Rehabilitation Research in the Clinical Sciences theme at MCRI, paediatrician in Developmental Medicine at The Royal Children's Hospital Melbourne and affiliated with the Department of Paediatrics, University of Melbourne.

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Brisbane site

Dr Priya Edwards is a Paediatric Rehabilitation Specialist in the Queensland Paediatric Rehabilitation Service, Children's Health Queensland Hospital and Health Service.

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Monash Medical Centre

A/Prof Michael Fahey is head of Paediatric Neurology at Monash Children's Hospital.

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The Children's Hospital at Westmead

Prof Nadia Badawi leads the CP Alliance Research Institute, is a neonatologist at The Children's Hospital at Westmead and affiliated with the University of Notre Dame Australia and the University of Sydney

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Associate Investigators

Associate investigators will be responsible for coordinating the team in their area of expertise or advising according to their expertise.

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Dr Katherine Lee, Associate Director of Biostatistics, Melbourne Children's Trial Centre, and senior biostatistician in the Clinical Epidemiology and Biostatistics Unit, Murdoch Childrens Research Institute

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Ms Janet Hough, senior physiotherapist. Monash Health; Victorian Paediatric Rehabilitation Service

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A/Prof Peter Anderson, psychologist, Group leader, Victorian Infant Brain Study, Clinical Sciences theme at Murdoch Childrens Research Institute

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Dr Kuang-Chih Hsiao, immunology fellow, Epworth HealthCare; honorary fellow, Murdoch Childrens Research Institute; and Clinical Lecturer at The University of Melbourne

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Dr Karin Tiedemann, transplant specialist (retired); Medical Director, BMDI Cord Blood Bank; Director, National Stem Cell Foundation of Australia

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Infusion

The infusion team will be headed by Dr Francoise Mechinaud, paediatric transplant physician at the RCH Melbourne. Dr Chris Fraser, LCCH Brisbane and by Dr Helen Savoia, paediatric transfusion medicine specialist, RCH Melbourne. Once the trial is underway, the steering committee will consider expanding the trial to include a third infusion site hospital, in NSW, to further demonstrate the feasibility of this trial as a multi-site project.

Therapy

All participants will receive their regular rehabilitation therapy.

Assessment

Safety assessment will primarily be conducted by both the infusion team (described above).Therapists and a psychologist independent of the therapy team providing rehabilitation, and laboratory staff, will complete other components of the safety assessment as well as assessments relative to secondary aims of the study. The team will undertake structured training in assessment tools prior to trial commencement.

Statistician

Dr Katherine Lee, located in the Clinical Epidemiology and Biostatistics Unit, Murdoch Childrens Research Institute.

1.3. Internal trial committees

Trial Steering Committee

Prof Dinah Reddihough (PI, Melbourne), paediatrician; Committee Chair
A/Prof Iona Novak (AI), occupational therapist
Prof Euan Wallace (AI), obstetrician at Monash Medical Centre
A/Prof Michael Fahey (PI, Monash), neurologist
Prof Nadia Badawi (PI, Sydney), neonatologist
Dr Katherine Lee (AI), biostatistician
Prof Mark Kirkland (AI), cord blood researcher
Dr Francoise Mechinaud (AI), paediatric transplant specialist
Dr Priya Edwards (PI, Brisbane), rehabilitation paediatrician
Prof Paul Colditz (AI), neonatologist
Dr Kylie Crompton (AI), scientist; Committee Secretary

Safety and Data Monitoring Committee

This committee will meet every three months, including after the first three participants have reached one month post-infusion for review prior to further participant infusions.

Prof Ross Pinkerton, paediatric oncologist (Chair)
Prof Rory Wolfe, statistician
A/Prof Adam Scheinberg, paediatric rehabilitation specialist
Dr Richard Mitchell, paediatric transplant specialist
Dr Catherine Marraffa, developmental paediatrician

Scientific Committee

Prof Euan Wallace, obstetrician
A/Prof Michael Fahey, neurologist
A/Prof Mark Kirkland, haematologist
Prof Paul Colditz, neonatologist
Dr Suzanne Miller, neuropathologist (The Ritchie Centre, MIMR-PHI, Monash University)
Prof Stephen Holdsworth, immunologist (Monash Health, Monash University)
Dr Ngaire Elwood, cord blood researcher

1.4. Funding and resources

Source	*Funding / Resource*
Research Foundation of the Cerebral Palsy Alliance	In kind; Cash

Cell Care Australia	In kind; Cash
All other participating institutions	In kind

2. INTRODUCTION AND BACKGROUND

2.1. Background information

Cerebral palsy (CP) describes a group of permanent non-progressive motor and postural disorders arising from damage to the developing brain while *in utero*, during birth or in the first years of life [1, 2], and affects around 2 per 1000 live births across the world. The main types of CP include spasticity (stiffness of muscles accounting for around 80% of all diagnoses), dyskinesia (abnormal involuntary movements) and ataxia (unsteadiness) which result from lack of normal nervous control of muscles. Depending on the location and severity of brain damage, different regions of the body may be affected. This is described as hemiplegia (one side of the body), diplegia (legs greater than arms) or quadriplegia (all limbs). The degree of motor impairment is often described using the Gross Motor Function Classification System (GMFCS), with GMFCS I indicating an otherwise normal child who may have impaired speed, balance or coordination, increasing in severity to GMFCS V indicating limited motor function, wheelchair use with limited head control. CP is often associated with epilepsy, difficulties in speech, sight, hearing, sensation, perception, behaviour or cognition. There is currently no cure for CP.

Scientific rationale

Recent interest in stem cell therapy for intractable neurological disorders has led to a large number of preclinical studies of brain injuries related to CP that show evidence of therapeutic potential. In particular, a greater understanding of the varied stem cells to be found in human umbilical cord blood (UCB), which is less ethically complicated than many other sources of stem cells, has led to a focus on UCB stem cell therapy. The stem cells in UCB do not form cancers and present a lower risk of graft-versus-host disease (GvHD) than bone marrow stem cells [3]. Transplantation of UCB cells in acute animal models of CP such as excitotoxic white matter injury [4] and neonatal hypoxia-ischaemia [5-10] have shown significant neurofunctional improvement, as have models of adult stroke [11-16], spinal cord injury [17-21] and traumatic brain injury [22]. On the other hand, preclinical studies showing no benefit from UCB cell (UCBC) treatment of acute brain injury have also emerged [23-25]. While some studies involve transplanting UCBCs directly to the injured area of the brain, there is strong evidence that the minimally invasive intravenous infusion to the periphery is equally as effective [4, 26]. Investigations into the mechanism of UCBC transplantation action reveal anti-inflammatory properties, protection of neural cells from secondary cell death, promotion of host cell proliferation and migration, and angiogenesis. It does not appear that human (xenogeneic) stem cells engraft to replace lost brain cells in immune-supressed animal models. Thus the stem cell treatment is not considered a transplant but can be thought of as a transfusion.

The transfusion mechanisms described above are appropriate for the acute phase of brain injury, which involves inflammation, primary and secondary cell death and chemical signalling. Preclinical work has almost entirely focussed on acute brain injury, and it is unknown if these mechanisms will operate in the same way in the chronic phase of disease. This area has been well reviewed in the literature [27-31]. CP is a heterogeneous condition with varied brain pathology, so it is possible that stem cell infusion may act through different mechanisms for different children. For example, some children have large brain lesions that might benefit from increasing the number of available neurons, whereas there is evidence that some infants born preterm may have reduced number of neural connections rather than reduced number of neurons [32]. Improvement in functioning for children with the former pathology may be due to recruitment of endogenous stem cells caused by UCBC infusion [33]. The latter pathology may potentially be linked to increasing synapses and dendritic arborisation rather than generating new neurons from either infused or endogenous stem cells [34]. Recruiting endogenous stem cells would take time to replicate, differentiate, migrate and integrate new cells, while generating new synapses from existing neurons *in situ* could occur sooner.

Safety discussion

Autologous blood transfusions are safe from the immunological point of view, and using cord blood rather than peripheral blood does not change the risk profile. There is an increase in risk when a cryopreserved rather than fresh blood product is used due to possible toxicity by residual dimethyl sulfoxide (DMSO) which can be reduced by 'washing' the cord blood unit prior to infusion. There is also a risk associated with any intravenous blood cell infusion that flooding the body with cells may temporarily block capillaries in the lungs. Both of these adverse events are temporary and treatable.

Infusing cells from anyone other than oneself introduces the risk of immune response. The first use of allogeneic UCBC infusion was a transplant in 1989 for a child with Fanconi anaemia [35] and 25 years later over 30,000 UCBC transplants have been conducted worldwide for children and adults with a range of haematological, immunologic and metabolic disorders. UCBC infusions conducted under coverage of immune-depleting conditioning regimen improves the likelihood of donor haemopoietic stem cells in the cord blood to persist and engraft in the recipient's bone marrow. After optimising the technique for 25 years, there is still a risk of mortality from GvHD, whereby the donor cells attack the immune-supressed recipient. This risk is reduced with improved HLA matching, and at its lowest using full matched related donors [3].

The preclinical data behind stem cell therapy as a possible treatment for CP demonstrates that donor UCBCs may not need to persist or engraft to mediate functional benefit. Given the risks and side effects, and little expected benefit, this protocol does not use a conditioning regimen or immune-suppressant. Without immune suppression, the recipient's immune system is expected to easily reject infused cells, further reducing the risk of GvHD. To minimise the risk of engraftment of UCBCs in participants with undiagnosed severe combined immune deficiency, clinical and laboratory screening of participants to exclude severe combined immune deficiency will be carried out.

The risk profile of the proposed UCBC infusion can perhaps be compared with a standard blood transfusion. There have been instances of transfusion-associated GvHD (TA-GvHD) from related donors, but in all cases these were shown to be in the specific case where the recipient was heterozygous for a haplotype that the donor was homozygous for (was haploidentical) [36, 37] – for example if the recipient's haplotype was MN, and the donor was MM, the donor cells recognise the host as foreign but the host does not recognise donor cells as foreign. This occurs more frequently in cultures with lower genetic variation such as Japan [38] and has led to the irradiation or leucodepletion of blood products from related donors [39]. Due to tissue typing and matching, this will not occur in this protocol and reduces the risk of GvHD relative to TA-GvHD.

A fraction of cells may remain in the body long term (microchimerism) as may happen after blood transfusion or pregnancy [37]. In studies of microchimerism after feto-maternal blood transfer, an association with auto-immune symptoms was found [40-42] which may possibly have relevance here. In a small study of long term microchimerism specifically looking for chronic TA-GvHD symptoms, no association was found although the level of evidence was low. When comparing cord blood with peripheral blood used in a transfusion, the cord blood has immature immune cells less likely to provoke a response than peripheral blood immune cells, but cord blood contains a larger proportion of stem cells compared with a peripheral blood transfusion which may have an impact on the degree of long term chimerism. Because there is no direct evidence of the longevity of matched sibling cord blood cells after infusion to an immune-competent recipient, we will use new, more sensitive, genetic analysis methods to follow the long term fate of cells to track risk of GvHD for individual participants, conducted by the Cytomolecular Diagnostics Laboratory, MCRI. This data, along with other safety assessments, will be monitored by the site transplant specialist.

The few relevant clinical studies of UCB stem cell therapy in patients with CP are described below. Clinical studies of CP must inevitably be in the chronic phase of disease and may not compare to acute preclinical studies. No efficacy studies using UCBCs in children with CP have yet been published internationally. The few studies available of UCB stem cell therapy in CP patients demonstrate safety and imply potential efficacy of this intervention, and are outlined below.

Case study – autologous cord blood

A case study of two toddlers in Thailand transfused with autologous (their own) UCB along with subcutaneous injections of granulocyte colony stimulating factor showed no side effects. The children improved as measured by the GMFCS [43].

Pilot trials – autologous cord blood

In a pilot study at Hanyang University Medical Centre, Republic of Korea, 20 children aged 2-10 years, with a range of CP types and born either preterm or term, were treated with infusion of their entire donated cord blood (rather than the nucleated fraction of cells as used in other studies). Cell numbers ranged from (0.6-15.65)x10^7/kg. Neurodevelopmental outcomes were monitored at 4, 8, 12 and 24 weeks and a range of neuroimaging studies were conducted at 24 weeks compared with a baseline measures prior to infusion. Functional improvements were seen in a range of activities in 25% of patients all of whom had hemiplegia or diplegia. No patients with quadriplegia showed definite improvements. There was some possible correlation between functional improvements and improvements on brain imaging; however the study was underpowered due to low number of patients [44]. Five patients (25%) experienced minor side effects, probably due in part to the inclusion of red blood cells in the infusion, which resolved with antihistamine and hydration. Despite its limitations, the study demonstrated practicality, safety and possible benefit of UCBC therapy [44].

Over the period 2004-2009, autologous UCBC infusions (>3x10^7 nucleated cells/kg infused) were performed at Duke University (Duke Blood and Marrow Transplant Program), USA, in children up to 8 years old with acquired neurological disorders, including 76% with CP [45]. No neuro-functional data was collected, but the infusions were shown to be safe and feasible, with only three adverse reactions across all 184 participants. These reactions were considered most likely to be due to residual dimethyl sulfoxide, DMSO, used in cryopreservation.

Randomised controlled trials – autologous cord blood

Subsequent to these studies which established the safety and feasibility of autologous UCBC infusion, randomised, placebo-controlled cross-over trials of autologous UCBCs commenced in children with CP. In 2010, Duke University (NCT01147653 [46]) and Georgia Regents University (NCT01072370 [47]), both in the USA, began trials involving 120 and 40 children and due to finish in 2016 and 2014, respectively. In addition, the University of Texas Health Science Center has recently started a comparative trial that aims to randomise 30 children with CP to a placebo arm and two treatment arms, comprising infusion of either autologous UCBCs or autologous bone marrow mononuclear cells (NCT01988584 [48]).

Randomised controlled trials – allogeneic unrelated donor cord blood

Bundang CHA Hospital, Republic of Korea recently published a trial of allogeneic (unrelated) cord blood combined with erythropoietin (EPO, a neuroactive potentiator), cyclosporine (a neuroactive immunosuppressant) and intensive rehabilitation therapy [49]. The trial used two control groups, the first received placebo blood, placebo EPO and immunosuppressant, the second received placebo blood and immunosuppressant but active EPO, with around 31 participants in each group (a total of 96 completed the trial). The cord blood treatment group was the only group to show benefit at 6 months after infusion compared with baseline, scoring higher on mental and motor assessment scales, which correlated with changes in diffusion tensor imaging (DTI). Sub-group analysis revealed that the younger children, and those with better-matched human leucocyte antigen (HLA) blood types, did the best with this treatment. There were no differences in serious adverse events between groups [49].

The Korean group is now examining allogeneic cord blood infusion combined only with immunosuppressant (NCT01528436, listed as complete in July 2012 [50] but unpublished, and NCT01639404 [51]). Two further trials have recently been listed on Clinicaltrials.gov, (NCT01991145 [52] and one which is focussed on analysing cytokine production, NCT02025972 [53]).

A recently listed randomised comparative trial is being conducted at the General Hospital of Chinese Armed Police Forces, China. Intrathecal infusion of mesenchymal stem cells derived from allogeneic cord blood will be compared with either one year of rehabilitation therapy or with normal clinical care, including 300 children aged 1-14 years randomised between the three arms (NCT01929434, [54]).

The accumulating preclinical evidence of the therapeutic value of UCB stem cell therapy, together with evidence from clinical trials for the safety of UCBCs from a range of sources, has led to increasing media coverage and has created hope and expectations in the CP community [55]. Yet the literature evidence regarding efficacy is mixed and clinical data are scarce. Trials to establish the efficacy of UCBCs in CP are complicated by the fact that, although autologous UCBCs are the safest type of stem cells, they are not available for most children with CP. On the other hand, unrelated donor UCBCs are readily available but pose a higher risk of graft-versus-host disease (GvHD). An alternative is matched sibling UCBCs which have the same level of major HLA match as autologous cells and have a decreased, although persistent, risk of GvHD relative to unrelated donor UCBCs. These are used clinically for children with haematological indications but, to our knowledge, have not been used for children with CP.

2.2. Rationale for current study

Despite the lack of conclusive evidence, UCBC infusion for CP is already in use in some parts of the world. Moreover, Australian children with CP are travelling to China, India, Germany and elsewhere to undergo UCBC therapy in an unregulated environment and at great cost. Therefore a well-designed and properly administrated trial evaluating the safety and efficacy of UCBCs in CP is necessary to guide clinicians and to inform patients and their families; and if successful, to develop treatment streams in Australia. Evidence from the previous studies outlined above suggests that autologous UCBCs may be ideal, and full matched sibling UCBCs the next best option. We have previously investigated the number of potential participants with autologous cord blood in storage. Although it may have been possible to conduct a trial with this group, numbers are small as children with CP are often born pre-term or with traumatic births that preclude cord blood collection. Sibling cord blood is more readily available as parents often store sibling blood following the diagnosis of a child with CP, and our partner Cell Care Australia is offering to cover the expense of prospectively collecting cord blood from siblings of children with CP to enable their eligibility to participate in this trial or future trials. Furthermore, during our feasibility assessments we met with representatives of the Australian Bone Marrow Donor Registry (ABMDR) which governs Australian public cord blood banks. ABMDR would not contemplate involvement in this research without demonstration of capability, and presentation of preliminary safety data ethically collected using a safer form of UCBC than unrelated donor UCBCs. Our long-term aim is to conduct a randomised trial to assess the efficacy of UCBCs from any donor in improving functional abilities of CP, to develop a treatment available to all children with CP. UCBCs will be infused intravenously as the least invasive delivery route. In order to give the brain the best chance of developing new pathways, rehabilitation therapy will also be used.

We hypothesise that the infusion of matched cord blood cells to a child with CP will induce a period of neuroplasticity that will be safe and may lead to functional benefit.

In the current study we plan to collect initial safety data. The results from this study will also provide a proof-of-concept in order to prepare and demonstrate capability for a number of possible future trials. This safety study will be investigate matched sibling UCBCs in children aged 1 to 12 years. In the future we would aim to conduct randomised trials to 1) assess efficacy of 12/12 HLA matched UCBCs for children with established CP compared with placebo; 2) assess the safety and efficacy of unrelated donor UCBCs for children with established CP with placebo and 3) assess the safety and efficacy of prospectively collecting CB at the birth of at risk children and re-infusing a) immediately if diagnosed with neonatal encephalopathy or b) within one year if signs of developing CP emerge compared with placebo. Future trials may focus on younger children since the limited evidence available indicates that younger children have the best chance of benefitting from UCBCs, however this age range has not yet been defined. We plan to use a wider age range of children for feasibility reasons and to help establish the best cut-point for future trials.

3. STUDY OBJECTIVES

Primary objective

The primary objective of this study is to gain preliminary information on the safety of 12/12 HLA matched sibling UCBC infusion in children with CP.

Secondary objectives

The secondary objectives of this study are:

A) to gain preliminary information on the treatment effect of 12/12 HLA matched UCBC infusion relative to baseline
B) to better understand the length of time that infused matched sibling UCBCs remain within recipients
C) to gather information and samples for future studies into mechanistic activity of UCBCs

4. STUDY DESIGN

This is a multi-site safety study.
Sibling donor UCBCs require careful DNA testing to ensure matching with the recipient (participant). Only about 25% of siblings have full-matched blood, the 75% that are not full-matched will be excluded. No immunosuppression will be used, as the infused cells do not need to survive for long periods within the recipient and immunosuppression increases the safety risk. The first six enrolled participants to receive infusions will be GMFCS IV or V; participants with mild or moderate CP (GMFCS I, II or II) will be waitlisted. This is because the safety profile of matched sibling UCBC infusion for children with CP is unknown, we feel it is more ethical to initially trial in the participants with severe CP and gather information for interim Data Safety Monitoring Committee (DSMC) review, before continuing the trial with less impaired participants. The DSMC will review safety data from the first three participants after three months post-infusion and decide whether the trial can progress to the next three participants. After these initial six participant infusions, the DSMC will decide whether the trial can progress to infusions of participants with mild CP or not. The DSMC will provide a report to the Trial Steering Committee, who will provide the report directly to the RCH HREC for review. The HREC will make a final decision about whether the trial can expand to include children with mild or moderate CP at this point, or whether the trial will remain restricted to children with severe CP only.

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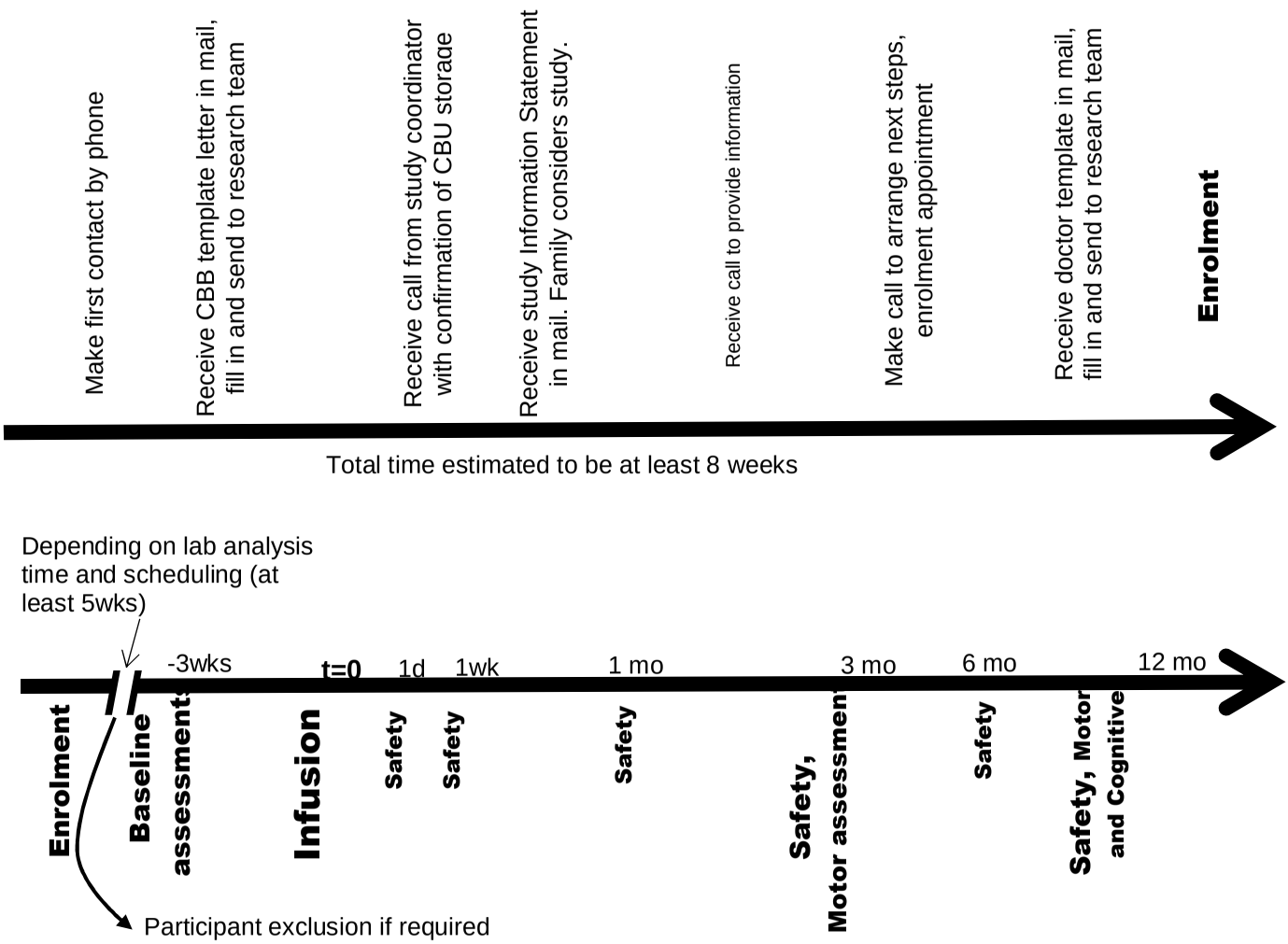
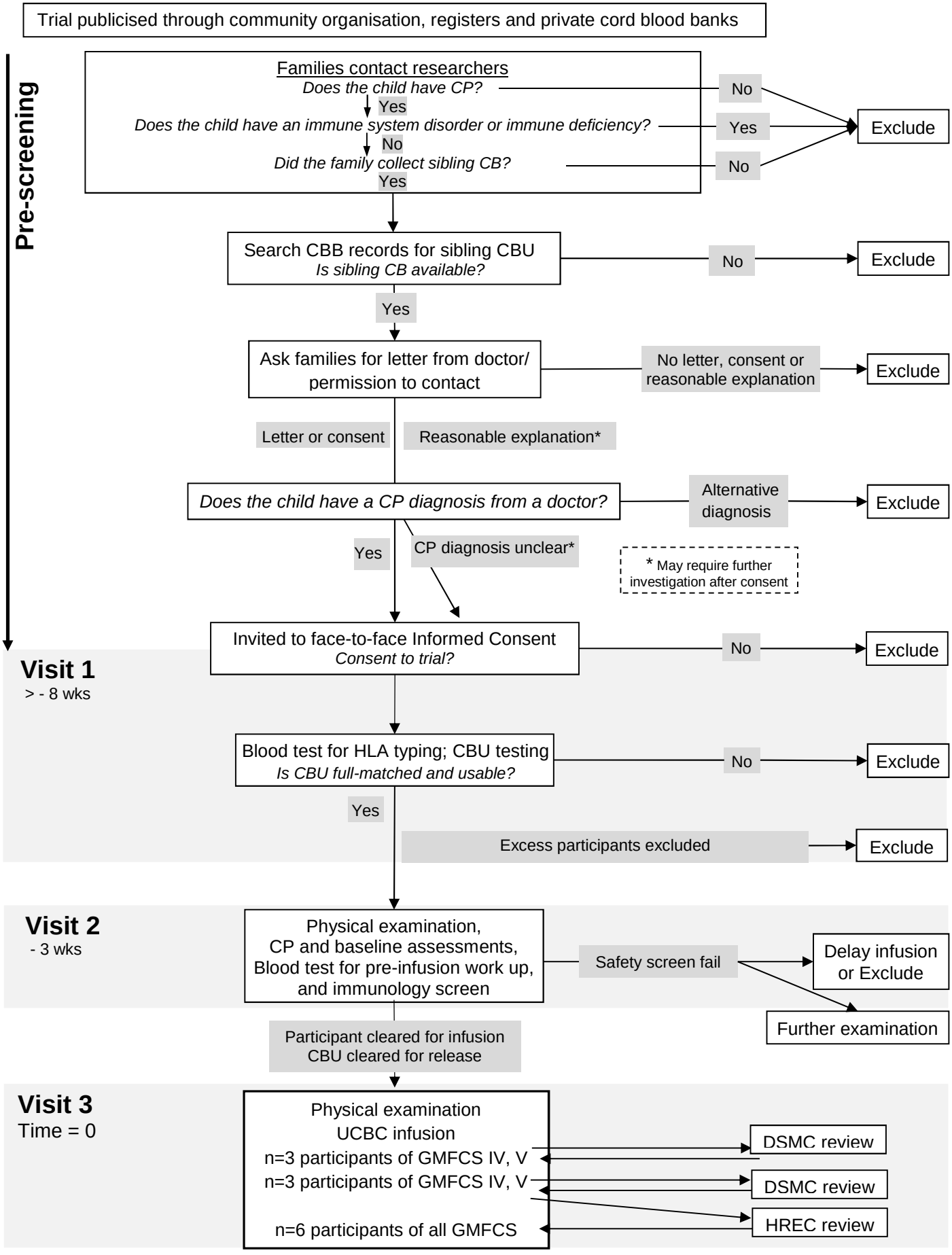


Figure 1: Participant timelines for the study

Figure 2: Recruitment decision making process (next page)


Trial publicised through community organisation, registers and private cord blood banks
Pre-screening
Families contact researchers
Does the child have CP?
Yes
Does the child have an immune system disorder or immune deficiency?
No
Did the family collect sibling CB?
Yes
No
Yes
No
Exclude
Search CBB records for sibling CBU
Is sibling CB available?
No
Exclude
Yes
Ask families for letter from doctor/ permission to contact
No letter, consent or reasonable explanation
Exclude
Letter or consent
Reasonable explanation*
Does the child have a CP diagnosis from a doctor?
Alternative diagnosis
Exclude
Yes
CP diagnosis unclear*
* May require further investigation after consent
Invited to face-to-face Informed Consent
Consent to trial?
No
Exclude
Visit 1
> - 8 wks
Blood test for HLA typing; CBU testing
Is CBU full-matched and usable?
No
Exclude
Yes
Excess participants excluded
Exclude
Visit 2
- 3 wks
Physical examination, CP and baseline assessments, Blood test for pre-infusion work up, and immunology screen
Safety screen fail
Delay infusion or Exclude
Further examination
Participant cleared for infusion
CBU cleared for release
Visit 3
Time = 0
Physical examination
UCBC infusion
n=3 participants of GMFCS IV, V
n=3 participants of GMFCS IV, V
n=6 participants of all GMFCS
DSMC review
DSMC review
HREC review

4.1. Number of participants

This trial will initially enrol up to 48 participants who fit initial eligibility criteria of having CP, no immune deficiency, and records of a CBU from the participating child or the child's siblings. This group will undergo detailed screening at visits 1 and 2 to determine further participation. Screening at visit 1 involves first line immunology screening (see section 7.1), tissue typing and cord blood unit examination of all participants to determine eligibility for trial groups. Screening at visit 2 involves general health screens. Enrolment will be staggered to allow sequential screening assessment and group allocation. Up to 12 of the 48 enrolled participants will continue with the trial after visit 2.

- Trial treatment and analysis will involve **12** participants with CP.

We have previously assessed the potential participant population by linking CP registers and cord blood banks (Victorian Cerebral Palsy Register, VCPR, with BMDI Cord Blood Bank, RCH HREC 32183; VCPR with Cell Care Australia, RCH HREC 32220; NSW CPR with Sydney Cord Blood Bank, ABMDR HREC 2012/06 and CP Alliance HREC 2012-12-04). We estimate that in early 2013 there were between 10 and 24 children over 2 years old with CP who had their own stored UCBCs across Australia. We do not expect that sufficient numbers would enrol in a trial of autologous cord blood infusion. Cord blood storage from siblings of children with CP increases every year, however we are unable to easily assess the number of children in Australia who have CP and sibling cord blood available. We have kept a register of families interested in this research that have a child with CP and sibling cord blood in storage and we predict that 15 (25%) of a predicted 60 suitable families across Australia are likely to be eligible for treatment. We believe sufficient numbers will prove to be eligible and will choose to enrol in the trial. The 12 treatment places will be filled chronologically from date of enrolment as participants proceed through screening. Once the 12 treatment places are full, excess participants will be excluded. To minimise this, potential participants will be told the approximate likelihood of continuing through the trial given the available number of places at the time of arranging a visit for informed consent. Potential participants will also be encouraged to continue any required medical programs irrespective of trial exclusion criteria, to prevent children delaying surgery or treatment with Botulinum toxin A while awaiting enrolment and then notification that they will either continue with the trial or be excluded. Trial infusion schedules will be staggered over six months to accommodate lab and space availability. Therefore, children who have received surgery or Botulinum toxin A treatments within the exclusion period can have their trial visits and trial treatments delayed to wait out the exclusion period if needed.

The remaining 75% of children who have CP and sibling cord blood available but do not have matching blood will be excluded from this trial. All excluded participants will be notified of the level of match or mismatch of their stored cord blood when they are notified of their exclusion.

Once all 12 participant places across sites have been filled, enrolment will cease.

4.2. Expected duration of study

The study is expected to take 2 years until final follow up and data analysis. This allows recruitment and screening over three months, then sequential treatment in groups of three. Each group of three is expected to be treated within one month, then followed until three months post-infusion for Data Safety Monitoring Committee (DSMC) review and approval to continue. After the first two groups of three, the final six participants will be infused. The final 12 month follow up assessment for each participant will occur 13 months after baseline measurements for each individual.

4.3. Primary and secondary outcome measures

The primary objective of this study is to collect initial safety data during and after infusion of sibling UCBCs. The following outcomes will be collected to assess this objective which will be assessed at three time points:

1) During the infusion and up to 36 hours after
    - Clinical assessment, including vital signs and pulse oximetry
    - Laboratory assessment looking for markers of infection

- Occurrence of AEs and SAEs

2) Within the three months after infusion
   - Clinical assessment
   - Laboratory assessment looking for markers of immune reaction
   - Deterioration of motor function as assessed by Gross Motor Function Measure
   - Occurrence of AEs and SAEs
3) Within the 12 months after infusion
   - Clinical assessment
   - Deterioration of motor or cognitive function as assessed by the appropriate tool (see section 7.3)
   - Occurrence of AEs and SAEs

The secondary outcomes include:

- Efficacy of the intervention at 3 months measured by therapists on a suite of standardised psychometrically sound measures of movement, cognition and quality of life. This will be assessed in an exploratory fashion only. The areas for assessment were selected to focus on motor outcomes because CP is a primarily motor disorder; on cognitive outcomes that have shown change in an international trial [49] and anecdotally from private stem cell clinics (changes in wakefulness, attentiveness and alertness have been described); and how these might affect the participant's life; The motor and quality of life outcomes will also be measured at 3 months. Cognition will be measured at 12 months after infusion and used to calculate change from baseline. Again, the analysis of the 12 month data will be exploratory in nature.
- Understanding the longevity of infused cells. This will be achieved using chimerism studies (engraftment analysis), looking for infused cells circulating in a sibling recipient's blood. We will study chimerism by extracting circulating cell free DNA from plasma and using a new quantitative PCR method detecting differences in copy number deletions between the recipient and donor. It will be reported as the fraction of donor DNA in the sample at each time point for each individual, and constitutes both a safety and mechanistic study.

The final objective of gathering information and samples for future studies into mechanistic activity of UCBCs will be achieved by collecting and storing serum, DNA and RNA from peripheral blood provided by participants before and after infusion as well as from the CBU. Serum will be used for cytokine analysis of participants post-infusion compared to pre-infusion. RNA arrays of neurotrophic and glial factors of UCBCs will also be compared with pre-infusion peripheral blood from the participant and with standard levels in adult peripheral blood.

4.4. Eligibility Criteria

Inclusion Criteria

To be eligible for this study, the following criteria must be fulfilled:

- Aged older than 1 year and younger than 16 years at time of enrolment
- Diagnosis of any type of CP
- CP of any severity
- A record of sibling CBU in storage at a TGA accredited private cord blood bank
- Ability to travel to one of the trial centres
- Ability to participate in assessments
- Informed consent by parent/guardian and an indication of willingness/compliance by children

Exclusion Criteria

Patients will be unable to participate in the trial if they:

- Show presence of progressive neurological disease
- Have a known genetic disorder
- Have a known brain dysplasia
- Have ever been diagnosed with an immune system disorder or immune deficiency syndrome
- Have infectious disease markers showing up on the virology screen

- The intended cord blood unit shows evidence of contamination, or has fewer than 10^7 cells per kg body mass
- Require ventilator support
- Are unwell, or if the participant's medical condition does not allow safe travel
- Have previously undertaken any form of cell therapy
- Have had, or are scheduled for, treatment with Botulinum toxin A within 3 months before or after infusion
- Have had, or are scheduled for, surgery within 3 months before or after infusion
- Cannot obtain parental or guardian consent

Participants must have CP. During the initial phone call, researchers will ask about a potential participant's CP, and inform families of the inclusion criteria. Nevertheless, as part of baseline assessments participants' CP diagnosis will be evaluated for eligibility.

CP is highly variable and it is not yet known whether all children with CP will respond to UCBC infusion in the same way. We will not exclude children based on the type of their movement disorder, on the area of their body that is affected or on the associated impairments that some children have. It is unlikely that children with extremely severe CP will gain greatly from this therapy, however, children with severe CP have the most to gain from the therapy, and our clinical experience is that these families are the most likely to risk travelling overseas for unregulated treatment. We considered that it would be unethical to ask a relatively unimpaired child to undertake unknown risk; however we feel that children with mild forms of CP have the potential to gain from the treatment as they do from other neuroplastic interventions [56, 57]. Therefore, this trial includes all levels of motor severity (GMFCS levels I-V), but groups the participant into bands according to severity – mild/moderate (GMFCS I, II and III) and severe (GMFCS IV and V). Participants with sibling UCBCs in storage will be scheduled according to severity with severe participants receiving infusions first. Safety data will be reviewed by the Data Safety Monitoring Committee after the first three (severe) participants, then after all severe participants have received infusions, at which point the DSMC report will be provided to the RCH HREC. If the HREC approve, mild/moderate participants will then receive infusions.

All evidence points to younger recipients receiving the most benefit from stem cell therapy, hence this trial is restricted to children 16 years or younger. A lower age limit is specified according to common age at diagnosis of CP, and development of a child's immune system; hence this trial is restricted to ages 1-16 years old.

At this point, only participants with cord blood units stored in private banks are eligible. If the ABMDR HREC and AusCord should approve this protocol, lead HREC approval will be sought to modify the protocol to include participants with publicly banked cord blood units.

It is possible that a participant may fit the exclusion criteria at enrolment or at some point prior to infusion. If, in the researcher's opinion, the participant may be eligible at a later date (e.g. if the participant were unwell), they will be referred to the site PI. The site PI will decide whether to remain in contact with the family for potential inclusion at a later date.

In this study we exclude children with known genetic disorders or brain dysplasias (congenital brain malformation due to abnormal neural migration during early foetal development) because we do not believe they are likely to respond to this therapy. However, we will not be conducting brain imaging or genetic testing and recognise the possibility that a child in this study may have an unidentified genetic disorder or brain dysplasia. We are excluding children who have previously had cell therapy as the previous therapy may continue to have an effect and make data difficult to understand, but as with genetic disorders and brain dysplasias, we will rely on self-report and acknowledge the risks of this.

4.5. Participant withdrawal

Participants are welcome to withdraw from the study at any time. Researchers will ask the reason for their withdrawal, and whether they are withdrawing from study treatments (UCBC infusion if they withdraw immediately after screening and baseline assessment) and/or the follow up assessments. If families agree to continue with the follow-up, researchers (either site PI or study coordinator) will contact participant families at each follow up assessment point by phone to arrange the assessment. Families that have previously withdrawn and have asked for no further contact will be excluded from this process. The timing and reasons for withdrawal will be documented in all cases.

Researchers may need to withdraw a participant for the participant's safety, for example if a serious adverse event occurs during infusion that prevents completion of the infusion. Additionally, if the study is terminated for any reason, all participants will be withdrawn. If participants require excluded medical procedures, such as surgery prior to the UCBC infusion, they will be withdrawn, but if the excluded medical procedures are required after the UCBC infusion the protocol violation will be recorded and reported but the participant will not be withdrawn from the study.

Regardless of when or why the participant withdraws, they will be offered medical assistance and follow up for the duration of the study to ensure the participant's safety.

5. STUDY PROCEDURES

5.1. Recruitment

In order to distribute information about this study, researchers will provide a description of the trial to CP registers and community organisations and to private cord blood banks (Appendix 1: Information to go in newsletters).

- Researchers will ask CP registers to publish details of this trial in their newsletters and invite families to contact researchers.
- Researchers will ask community organisations to distribute the information as appropriate.
- The private cord blood banks will notify their clients of this trial and invite families to contact researchers.

Recruitment through clinicians and allied health professionals will be welcomed. Our experience is that there are many families who are interested in stem cell therapy and have brought this to the attention of their clinicians. Researchers will therefore make information available by providing the Australasian Academy of Cerebral Palsy and Developmental Medicine (AusACPDM) with information to circulate to clinicians and allied health professionals working in the field of CP. Thus, if families approach their clinicians and allied health professionals to discuss the trial, the professionals will have some understanding. Making information available for health professionals will also be valuable for later in the trial, particularly in areas of Australia far from infusion sites.

Families that contact the study team will be verbally informed about the trial by the lead site study coordinator, will have the opportunity to ask questions and will be informed of eligibility requirements. Researchers will ask for verbal consent to record some details about the potential participant in the study database (does the child have CP, do they have an immune deficiency, did they store cord blood at birth of any child, which child, where, family contact details; see Appendix 2: Script for first contact phone conversation, Appendix 3: Record of verbal consent, and Appendix 4: Letter following verbal consent).

If families believe they donated the potential participant's sibling UCB, and they would like to find out if the CBU is recorded at a CBB, researchers will mail the family a template letter (Appendix 5: Template to ask CBB to locate CBU records) which explains the purpose of the requested CBU search, has space for the family to fill in required details including the participant's sibling's date of birth, hospital of birth, sex and mother's details, and a signature constituting consent for the CBU search and for the CBB to provide researchers with results of the CBU search. A second copy of the template letter will be included for

families to retain as a record of their consent, and a reply-paid envelope for families to mail the first copy to researchers (rather than directly to a CBB, to preserve CBB independence from the trial). Once researchers receive this template letter as consent, the trial coordinator will provide the appropriate CBB with a copy so that they are able to locate a record of the CBU. Families will be notified of the outcome of the record search by phone. The details from those who verbally consented during the initial phone call, and signed consent following the record search will be recorded in the study database as part of initial recruitment data.

Once the CBU record search is complete, the lead site study coordinator will phone to provide the results of the search, to discuss the next steps with potential participant families (Appendix 6: Script for phone conversation after CBU record search, or part of the above phone call if appropriate) and to encourage families to discuss the trial with their clinician. An Information Statement without the consent form will be mailed to families so that families can continue considering their involvement in the trial. Families will be able to contact the lead site study coordinator or researchers to ask questions and discuss the study. If they choose to enrol, they must contact the lead site study coordinator or researchers to arrange an initial visit to an infusion hospital for the participant and family for informed consent. During this phone contact, the trial coordinator will inform families of the current number of places available within the trial and will estimate the likelihood that the potential participant will continue through the trial, and will request confirmation of the child's diagnosis (Appendix 7: Script for phone conversation to arrange Visit 1, Appendix 8: Letter to family to request confirmation of diagnosis).

If needed, and if the family consent, a letter and copy of the signed form (Appendix 8:) will be mailed to the nominated child's doctor asking for information (Appendix 9: Letter to child's doctor requesting information). If a family does not provide documentary evidence of CP diagnosis or consent to contact with a diagnosing or treating doctor, they may discuss it with the study doctor. If the family do not provide a reasonable justification for the lack of CP diagnosis, the child may be excluded. If the family provides reasonable justification, the child may require assessment by a paediatrician after Informed Consent, but prior to other study procedures. This will be at the discretion of the study doctor.

A record of all participants that were pre-screened or enrolled by researchers will be sent to and kept by the lead site study coordinator, including reasons for ineligibility or non-participation if available, to inform future trial design. All participants who do not receive the intervention will also be asked whether they would like to be contacted about any appropriate future trials, and they will be informed that future trials may focus on younger children. The custodian of this databank will be the lead site study coordinator at the lead site until the conclusion of the trial, at which time custodianship will shift to the coordinating principal investigator.

5.2. Enrolment (visit 1)

At this visit, informed consent will be conducted face-to-face with the parent or legal guardian of the intended participant and the legal representative of the cord blood, either in person or by Skype or other video conferencing with the trial coordinator or a trial investigator. In most cases, because the intended participant and the cord blood donor must be full biological siblings they will have the same parent/guardian who is also the legal representative of the privately stored cord blood. Treating clinicians will not be able to take informed consent.

Participants will then give a blood sample for tissue typing to match with CBUs. The samples will be collected either by the pathology collection service at the infusion hospital or at a local pathology collection service, and then blood for tissue typing will be sent to the transplantation services division of the Australian Red Cross Blood Service appropriate to the participant's location. They will undertake generic HLA-A, HLA-B and HLA-DR (which are inherited together and called a haplotype; each genome has two haplotypes) matching with CBUs to confirm that the identified sibling CBU is a match with the participant. Note that if the generic testing of HLA reveals that both haplotypes match, i.e. 6/6, inheritance of HLA alleles as entire haplotypes means that 12/12 HLA alleles match but nevertheless we will conduct tissue typing to assess 12/12 HLA match. Tissue typing data will be received by a transplant physician at each site. If the CBU is a match, the transplant physician will order CBU pre-release work up from the CBB. ABO group and rhesus type will be checked at this time for participants who will receive sibling UCBCs, but incompatibility will not

prevent infusion. Sibling ABO group and rhesus type will be analysed from the CBU if sufficient red blood cells are available; otherwise it will be accepted from hospital records if available. If neither of these methods reveal the sibling ABO group and rhesus type, the sibling will need to attend the infusion hospital and provide a blood sample for testing.

If the participant has more than one HLA matched sibling CBU available, determining which is selected for the trial will rely on ABO Rhesus matching. The presence of multiple sibling CBUs in storage will be established prior to enrolment, and extra blood will be collected from the participant during visit 1 to determine the participant's ABO Rhesus group.

Both the participant and the sibling donor will need to provide saliva samples for genotyping prior to copy number deletion chimerism assays. These samples can be collected at the hospital or the trial coordinator can provide the family with collection kits which can be returned at visit 2.

5.3. Group allocation

This study involves only a single group. If an individual participant has multiple eligible cord blood units in storage, a full matched sibling unit with matching ABO group will be selected as first preference and full matched sibling unit with mismatched ABO group as second preference.

Around 75% of enrolled participants will be excluded because their sibling UCBCs in storage are not a full match. These participants will be notified immediately, but participants with matched CBUs who are excluded due to a full treatment group will not be notified immediately because visit 2 involves further screening that may require replacement participants. Once all continuing participants have completed visit 2, excluded participants will be phoned by either the site PI or the trial coordinator to communicate their exclusion in the research study. At the conclusion of this study, we will send them a summary of results if they would like that.

Some children may have epilepsy given it is a common association with CP. Regardless of past history of epilepsy, participant families will be required to keep an epilepsy log to record frequency and severity of epileptic events to compare with a similar log after infusion (data collection form is attached).

Families will be referred to counselling services if they feel distressed by learning of their exclusion. Families may feel upset to learn that their child will be unable to use the cord blood unit in storage. Families may also be upset at the conclusion of the trial if they did not perceive any improvement during the trial.

5.4. Baseline (visit 2)

Participants will be required to attend a second visit at the infusion hospital prior to infusion to confirm eligibility and establish baseline assessments. Participants will meet the trial doctors (both paediatrician and transplant specialist) to provide a medical history (including Appendix 10: Immunological screening questions) and undergo a physical examination including a photo of the participant's skin to record characteristic colouring and evidence of rash at baseline, the trial therapist who will conduct a CP assessment, and the therapist and a psychologist to establish baseline assessments. If clinical screening reveals a temporary issue, such as having a current infection, which may affect results of a screening immunology test, the participant may need to return to the hospital at an appropriate time in the future before continuing further.

The participant will then provide a blood sample at the hospital pathology collection service which will be used for confirmatory HLA typing (blood sent to Red Cross transplantation service for tissue typing of 6 HLA alleles), virology (blood sent to Red Cross Blood Service for nucleic acid test (NAT) HIV 1 & 2, anti-HIV 1 & 2 antibodies, Hepatitis B surface antigen, anti-Hepatitis B core antibody, NAT Hepatitis C virus, anti-Hepatitis C virus antibody, anti-Human T-lymphotropic virus 1and 2 antibody and syphilis testing) and other pre-infusion screens through the hospital laboratory services to ensure the participant is otherwise healthy. The participant's immune-competence will be screened at this visit, with full blood examination, lymphocyte subsets, naïve T cells and immunoglobulins. The results will be interpreted along with the participant's clinical history and responses to the immunology screening questions. Trial doctors

will exclude from the study any participants suspected to have an immune deficiency disorder and refer them to the local clinical immunology team for further evaluation and management.

In some cases, it may be necessary to collect blood from the participant's mother, if Hepatitis B core Antibody (HepBcAb) was not tested from her blood at the time of cord blood collection, and depending on HepBcAb results from cord plasma sample tests. This allows a full risk assessment of HepB contamination. Clinically, HepBcAb positive cord blood that indicates a previous (maternal) exposure to HepB but no current infection would be eligible for use, however in this research context we cannot take even this minimal risk of contamination.

This visit must occur a minimum of five weeks after Visit 1, to allow time for HLA typing, the CBU order to go to the CBB and three weeks for a private CBB to complete release work up including contamination assessment on the CBU and transport of CBU to the cell therapy lab of the infusion hospital. The infusion hospital needs a minimum of three weeks between arrival of the CBU and infusion, to finalise release assessments. The virology and immunology screen during Visit 2 must occur between 10 and 30 days prior to infusion at Visit 3. Visit 2 will be scheduled accordingly.

5.5. Infusion (visit 3)

Following the confirmation of CP, participants will be invited for a third visit at the infusion hospital where they will undergo their infusion. All CBBs in Australia process cord blood to reduce volume, deplete the concentration of red blood cells and collect the buffy coat which includes all nucleated cells and a fraction of red blood cells (most of which lyse upon cryopreservation). There are minor differences in CBB protocols such as the designated minimum volume of cord blood and the minimum of total nucleated cells (TNC) in the cord blood eligible for storage. As far as possible, UCBC treatment will be kept uniform among participants. If a record search identified a CBU and the participant enrolled in the study, HLA type of the CBU will be compared with HLA type of the participant to ensure it is a 12/12 HLA match. AusCord CBUs have already been HLA typed and their records will be used; private CBBs will send a pilot sample of the CBU to the Red Cross transplantation service for HLA typing. If the CBU is correct, all CBBs will undertake a reference thaw if not already completed, to test the quality of the CBU and confirm tissue type. Next, the transfusionist will organise CBU transport to the infusion hospital cell therapy lab where it will be stored below -180ºC in the vapour phase of liquid nitrogen. On the day of infusion, lab staff will thaw the CBU and then wash by diluting in dextrose/albumin and centrifuging to collect cells to minimise the presence of DMSO cryopreservant (less than 1% DMSO remains, an undetectable scent). The infusion hospital labs will report on post-thaw CBU qualities after release, including TNC, cell viability, CD34+ cell count, aerobic and anaerobic contamination and supernatant haemoglobin (see section 7 for details).

Upon arriving at the infusion hospital day medical facility on the day of the infusion, the participant will provide a blood sample to the research nurse. This sample will have DNA, RNA and serum proteins extracted to compare with CBU and with post-infusion blood.

Cannulation A peripheral vein catheter (PVC) will be inserted in the foot, hand or antecubital fossa after application of a numbing cream.

Age range	Gauge of PVC
Infants and toddlers	24 – 22
Older children	22 – 20

Hydration: All participants will receive intravenous hydration, starting 2 hours prior to infusion and continuing for 4 hours after the conclusion of the infusion. This is to ensure good clearance of any residual red blood cells and free haemoglobin following infusion. Hydration will continue until urine is clear and there is no indication of hypertension.
0.9% NaCl (normal saline)
125 mL/m^2/hr for a total of 6 hours
Ordered by transplant physician or delegate
Infused by nurse

Pre-Medication Ordered by transplant physician or delegate
Administered by nurse

Medication	Administration	Timing	Reason
Hydrocortisone 25-100 mg	Intravenous	2 hours prior to infusion	To prevent allergic reaction
Antihistamine*	Oral or intravenous	1 hour prior to infusion	To prevent allergic reaction
Paracetamol	Oral	1 hour prior to infusion	Pain prevention
Ondansetron	Oral	1 hour prior to infusion	Nausea prevention

* The antihistamine will be selected according to clinical indication. Promethazine (Phenergan), which has sedative properties, may be used for children with saliva and airway control, but if there is concern about the airway cetirizine (Zyrtec) or other antihistamines may be used.

Infusion: Intravenous (foot, hand or antecubital fossa)
Catheter size as above (compromise between smallest being easiest for children, whilst remaining large enough to prevent cell damage)
Infused over the course of 10-20 mins depending on age of the participant
Maximum volume of infusate is 10 mL/kg recipient
Ordered by transplant physician or delegate
Infused by nurse
Additional infusion identity check by study staff

Minimum TNC: $> 1 \times 10^7$ cells/kg body mass (in line with other international trials)

In the case of a Rh+ donor and Rh- recipient, the transfusionist or transplant physician will order anti-Rhesus D immunoglobulin (Anti-D) from the hospital blood bank, which will be delivered by i.v. or i.m. injection 24 hours after infusion (at visit 4) to prevent development of antibodies. The Anti-D administration is precautionary, the red blood cell depletion of CBUs and lysing of remaining red blood cells during cryopreservation combined with the CBU washing procedure means there should be negligible red blood cell antigens (ABO, rhesus, Kell or others) remaining.

All participants will be observed carefully by the nurse following the infusion (see section 7) with a transplant physician available if observations fall outside the normal range (see section 8). Blood will be collected by the nurse 1 hour after conclusion of infusion to compare with baseline.

Participants will be released from the hospital if they are haemodynamically stable and do not have haemoglobinuria. Participants will return to the hospital the next day for a physical examination and blood test. If there is any indication of infection or serious adverse event, the participant may need to remain in hospital for observation or medical attention. This is not expected. Participants will be provided with emergency phone numbers and instructions (see Appendix 11: Post-infusion handout).

Further blood samples will be taken at 1 week, 1 month and 3 months, for both research and safety reasons (see Table 1).

Table 1: Details of all proposed blood samples

Blood collection	*Volume*	*When is it taken?*	*What is it for?*	*Where does it go?*	*What happens to it afterwards?*
1	9 mL; Blood spot	Soon after enrolment	- Low resolution HLA typing to match sibling or autologous CBU with participant - Immunology screening	- Red Cross Transplantation Services - RCH Melbourne Immunology Lab*	Transported, destroyed

Sibling sample	2.7 mL	Soon after enrolment (only if assay cannot be conducted on CBU)	- To assess blood group and rhesus type	- Infusion hospital labs	Transported, destroyed
Maternal sample	2.7 mL	3 weeks prior to infusion (if not previously conducted)	- To assess HepBcAb (to assess risk of HepB contamination of cord blood)	- Infusion hospital labs	Transported, destroyed
2	9 mL 7 mL	3 weeks prior to infusion	- Low resolution confirmatory HLA typing to ensure matching blood is used - To assess blood group and rhesus type - To assess health of participant	- Red Cross Transplantation Services - Infusion hospital labs	Transported, destroyed
3	4 mL 7.2 mL	2 hours before infusion	- To compare with the blood sample taken after treatment	- Cell therapy lab - Infusion hospital labs - Cyto-molecular diagnostics lab* - Research labs*	Frozen, transported and stored
4	8.2 mL	4 hours after infusion	- To look for inflammation - To compare with blood taken at baseline	- Infusion hospital labs - Cyto-molecular diagnostics lab* - Research labs*	Frozen, transported and stored
5	8.2 mL	24 hours after infusion	- To check for infection - To check the cell types in the participant's blood	- Infusion hospital labs - Cyto-molecular diagnostics lab* - Research labs*	Frozen, transported and stored
6	8.2 mL	1 week after	- To look for inflammatory markers, signs of GvHD - To check the cell types in the participant's blood	- Infusion hospital labs - Cyto-molecular diagnostics lab* - Research labs*	Frozen, transported and stored
7	6.4 mL	1 month after	- To check the cell types in the participant's blood, chimerism assessment - To look for inflammatory markers, signs of GvHD	- Infusion hospital labs - Cyto-molecular diagnostics lab* - Research labs*	Frozen, transported and stored
8	6.4 mL	3 months after	- To check the cell types in the participant's blood, chimerism assessment - To look for inflammatory markers, signs of GvHD	- Infusion hospital labs - Cyto-molecular diagnostics lab* - Research labs*	Frozen, transported and stored

* Centralised testing, involving sample transport to Melbourne. RCH Melbourne Immunology Labs will undertake first line immunology screening; MCRI Cyto-molecular Diagnostics Lab will undertake chimerism assays.

5.6. Rehabilitation therapy

Standard care for children with CP involves the provision of rehabilitation therapies. It has also been hypothesised that stem cell-induced neuroplasticity might be optimised under "enriched" conditions, i.e. in the presence of activity-dependent plasticity arising from active rehabilitation based on neuroscience principles [58]. For both ethical and scientific reasons, all participants enrolled within this study will continue to receive their standard rehabilitation treatments. The study will request a monthly home diary of the family-determined therapy regimen for the duration of the trial.

6. STUDY VISITS AND PROCEDURES SCHEDULE

Study phase	*Enrolment*	*Baseline*	*Infusion*	*Follow up*					
Visit number	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*
Timing	*> 8 wks prior to infusion*	*21 days prior to infusion*	*0*	*1 day*	*1 wk*	*1 mo*	*3 mo*	*6 mo*	*12 mo*
Tolerance	*Any time*	*> 3 wks since Visit 1*	*- Within 10-30 days of clear virology screen* *- CBU available*	*± 0 days*	*±2 days*	*± 7 days*	*± 2 wks*	*± 2 wks*	*± 2 wks*
Location	*Hospital*	*Hospital*	*Hospital*	*Hospital*	*Hospital, home*	*Hospital*	*Hospital*	*Hospital, community*	*Hospital*
Informed consent (45 mins)	X								
CP assessment (30 mins)		X							
Medical history (0.5 hr)		X							
Physical examination (0.5 hr)		X	X	X		X	X	X	X
Medical assessment (10 mins)				X	X	weekly	X	X	X
Assessment of motor function (45 mins)		X					X		X
Assessment of upper limbs (15 mins)		X					X		X
Assessment of quality of life (0.5 hr)		X					X		X
Assessment of cognition (2hr 15)		X							X
Infusion of UCB (30 mins)			X						
Intravenous fluids, observation (6 hrs)			X						
Anti-D administration if required (5 mins)				X					
Blood collection	X	X	2X	X	X	X	X		
Rehabilitation Therapy									
Length of visit	1 hr	6 hrs (may need two visits)	9 hrs	1 hr	1 hr	1 hr	2 hrs	1 hr	4 hrs

7. CLINICAL AND LABORATORY ASSESSMENTS – METHODOLOGY

7.1. Safety measures

Medical History

A medical history will be taken at visit 2, and permission to access hospital medical records is requested.

A questionnaire will be used to gather information on immunological health status.

Physical examination

Physical examinations will be conducted by both a paediatrician and a transplant specialist prior to infusion. They will examine the participant to confirm eligibility by confirming the CP diagnosis during visit 2, and to ensure they are healthy. Participant weight and height will be measured and BMI calculated, the doctor will listen to the heart and lungs, feel the abdomen, take pulse and respiratory rates and confirm the neurological signs including assessing muscle tone and reflexes.

After infusion, a transplant specialist will physically examine each participant at visits 4, 6,7 and 8 (1 day, 1, 3 and 12 months post-infusion) as part of the medical assessment, to look for subtle signs of GvHD that may not be picked up by participant carers.

Medical assessment

Medical assessment will be conducted at 1 day, weekly until 1, 3 and 12 months after infusion, and at any time that the participant or their family feel concerned. All medical assessments will be conducted by a transplant specialist. It comprises a short list of questions of participant safety over the phone from the trial doctor, focused on signs of infection and inflammation. Should the participant show any indication of needing more detailed examination, they will be asked to return to the infusion hospital as soon as possible. If necessary, they may of course attend their nearest medical service and the trial doctor will follow this up.

At visit 6 (6 months post infusion), a paediatrician either at a trial site hospital or in the local community will conduct a general health assessment of the participant.

Observation

Vital signs will be monitored by the nurse prior to UCBC infusion, during the infusion, every 15 minutes for 1 hour, then every 30 minutes for 2 hours. The monitoring will include temperature measurement (in ºC), blood pressure (in mmHg), pulse oximetry (in % oxygen saturation) and checks for nausea and discomfort.

Participants will also be monitored for symptoms of toxicity, including nausea, fever, chills, and for symptoms of anaphylaxis including wheezing and urticaria.

A transplant physician will be available and called if vital signs fall outside the normal range (see section 8) or if there are signs of an adverse event.

Assessment of CBU

Cord blood is assessed initially upon donation prior to cryopreservation, including a maternal health follow up. While preparing to release a CBU, the CBB will undertake a reference thaw of a pilot sample to predict post-thaw characteristics, and a pilot sample will also be sent to the Red Cross Transplantation Service for HLA typing. If the CBU meets quality and release criteria of the CBB and fits eligibility requirements at this point, it will be transported to the cell therapy lab at the infusion hospital.

The cell therapy lab of each infusion hospital will examine the CBU for release. If the CBU passes inspection again, the infusion date will be confirmed. On the day of infusion, the CBU will be thawed and washed and samples taken to assess for TNC, CD34+ cell count and viability, aerobic and anaerobic sterility, and supernatant haemoglobin although results from these assays are not available until a day or more after infusion. This follows standard operating procedure in preparation for haemopoietic stem cell

(HSC) transplant and hence focuses on HSCs. This is useful information and will form part of the study report; however UCBC therapy for CP has quite different aims to a HSC transplant.

Therefore, the private cord blood bank labs will also conduct assessments of UCBCs with a focus on other cell types and potential modes of activity. Immunophenotyping will examine the range of cells present; and monocyte activity will be examined.

Analysis of blood – pre-transplant work up

Blood samples taken within 3 weeks of infusion will be used to assess general health and eligibility for infusion. A full blood examination, blood group and antibody testing, urea, electrolyte and liver function tests, C-reactive protein assay and virology screen will be undertaken.

Analysis of blood – immunology screen

The immunology screen involves full blood examination, immunoglobulin levels, lymphocyte subsets and naïve T-cells (or the local laboratory's equivalent test) performed at the local hospital's diagnostic laboratory. Local diagnostic laboratories' reference ranges will be used to interpret results in conjunction with the participant's clinical history and responses to the immunology health screening questionnaire.

Analysis of blood – studying infusion safety and mechanism

Blood samples taken immediately before, 4 hours, 24 hours, 1 week, 1 month and 3 months after infusion will be used to collect serum, DNA and RNA.

The sample taken prior to infusion will be used as both a baseline for any changes after infusion, as well as a control sample to compare with each participant's CBU.

The samples taken up until 1 week post infusion will have a full blood examination, urea, electrolyte and liver function tests, C-reactive protein assay and lymphocyte subsets assay. Chimerism will be assessed using a new digital PCR method to detect copy number deletions (CNDs) which is more sensitive and accurate than current standard chimerism assays (sensitivity to 50 genome equivalents/mL). This assay will be centralised to MCRI Cyto-molecular Diagnostics Lab as it is not generally available, and therefore requires sample transport. These assays are for safety, and to examine longevity of infused sibling cells in the circulation.

This study will also gather information on the activity of infused cells. The focus will be on immunophenotyping, to look at populations of classically (M1) or alternatively (M2) activated macrophages, T_{reg} cells and other immune cells, since this is one of the primary modes of UCBC action.

The samples taken at 1 and 3 months after infusion will be used for C-reactive protein assay and lymphocyte subsets assay. These are to examine longevity of infused cells, and for safety studies to determine whether infused cells have engrafted, whether chimerism can be detected from participants receiving matched sibling UCBCs, and include assays for inflammatory markers. These tests will monitor and determine the risk of GvHD for the participants. If chimerism is still detected at 3 months, further testing may be needed.

A portion of the samples will be stored at -80ºC for future studies. This is important, as we expect a far greater understanding of the activity of UCBCs in cerebral palsy to emerge over the coming years which would guide future research.

7.2. Descriptive measure (CP assessment)

The participant's CP will be described during visit 2, using the Australian Spasticity Assessment Scale (ASAS), Manual Assessment Classification System (MACS) and the Gross Motor Function Classification System (GMFCS) and the Communication Function Classification System (CFCS). In addition, the presence of active epilepsy, controlled epilepsy or no epilepsy will be recorded. Other eligibility criteria will be addressed during the physical examination.

7.3. Evaluative measures

Evaluative measures will be taken at baseline prior to infusion (visit 2) and after intervention at follow-ups visits defined below. A copy of the assessment results at baseline and final assessment will be available for families if they would like them.

Motor assessment (baseline and at visits 7 and 9)	
The gold standard gross motor measure for CP, the Gross Motor Function Measure (GMFM-66) will be used. The GMFM-66 has good validity and reliability, and is the only gross motor measure responsive to change for children [59]. The assessment can be scored from videotape if needed.	45 mins to complete (participant)
An upper limb assessment will also be used, the Quality of Upper Extremity Skills Test (QUEST). This tool is validated for use with children aged 18 months to 8 years and has been used in the older range needed for this study [60]. Results will be reported for each limb separately. This tool is reliable and sensitive to change, but has the disadvantage that it measures impairment reduction rather than functional improvement. The QUEST can be scored from videotape if needed.	15 mins to administer (participant)
Quality of life assessment (baseline and at visits 7 and 9)	
Assessors will use the Cerebral Palsy Quality of Life Questionnaire (CP-QOL) for validated for adolescents 13-18 years old, or for children less than 12 years the CP QOL-Child. This measures social wellbeing and acceptance, feelings about functioning, participation and physical health, emotional wellbeing and self-esteem, access to services, pain and impact of disability and family health. Some of these items are not expected to change over the course of this study. It is completed by the participant's caregiver, and also by the participant for 9-18 year old [61, 62].	15-25 mins to complete (parent and participant)
Cognitive assessment (baseline and at visit 9)	
No specific cognitive training programs have been validated for use with children with CP, however the limited evidence available indicates there may be some spontaneous improvement in cognition. Therefore, cognition will be assessed by a psychologist in an age appropriate manner (see Table 2 below), ensuring that an individual is tested using the same tool before and after the intervention. There is a possible learning effect with cognitive assessment tools, in that using them too often leads to familiarity with the tool which confounds data on actual change in cognition, therefore most tools are recommended for use only every 12 months. Therefore, we will avoid assessing cognition at the 3 and 6 month assessment visits but will assess at baseline (-1 month) and 12 months. Note that this assessment may not be possible for children with extremely severe CP.	1 hour to administer (participant)

Table 2: Cognitive assessment tools for each age range.

Age Range (at baseline)	Assessment tool
1-2.5 years (validated 3-42 months)	Bayley Scales of Infant and Toddler Development 3rd Edition (Bayley-III)
>2.5-6 years (validated 2.5–7 years)	Wechsler Preschool Primary Scale of Intelligence (WPPSI–IV)
>6 years (validated 6-16 years)	Wechsler Intelligence Scale for Children (WISC-IV, or WISC-V if released prior to trial commencement)

The Vineland Adaptive Behaviour Scales (Vineland-II) assesses personal and social skills needed for everyday living and is validated for use across the lifespan (birth-90 years). This assessment will be used in the parent/caregiver reporting format for all participants, to allow use for children with severe CP who would not be able to complete the assessment on their own behalf.	30 mins to administer (parent)
The Behaviour Rating Inventory of Executive Function (BRIEF) will be used to provide information on how problems such as attention difficulties, poor working memory, disinhibition and planning/organisational impairments impact everyday behaviour. The BRIEF is used for 5-18 year olds, the preschool version BRIEF-P is used for 2-5 year olds. It is a parent questionnaire of 86 items.	15 mins to administer (parent)
The Strengths and Difficulties Questionnaire (SDQ) is a brief series of 25 questions for parents which, although broad, correlate well with more detailed assessment tools. A set of Australian norms are available. This tool can be used for 2-16 year olds (parents of 2-4 year olds receive a slightly modified version).	5 mins to administer (parent)
The Beery-Buktenica Developmental Test of Visual-Motor Integration (Beery VMI) is a virtually motor-free assessment for children.	15mins to administer (participant)

8. REPORTING ADVERSE EVENTS

Definitions

An adverse event (AE) is defined as any untoward medical occurrence in a patient enrolled in this study, whether due to study treatment or not. All AEs will be recorded and evaluated by study doctors, and their relationship to the treatment will be assessed. AEs are limited to those that initiate or worsen during the course of the trial. AEs will be categorised by severity according to Appendix 13: Categorising severity of AEs related to study treatments: NCI Common Terminology Criteria for AEs (CTCAE).

A serious adverse event (SAE) is an AE that is life threatening; may require immediate hospitalisation or transfer to intensive care unit; results in persistent or significant incapacity or even death. It also includes any AE that requires immediate medical intervention to prevent any of these listed consequences. Severity grades 4 and 5 will automatically be designated as a SAE, some discretion by the study doctor may be required for grade 3.

A suspected unexpected serious adverse reaction (SUSAR) is any unexpected SAE that is suspected to be related to the study treatment. For example, infusion-associated graft-versus-host disease (GvHD) and anaphylaxis are potential risks that may be classified as SUSARs.

Eliciting Adverse Event information

Adverse events will be recorded from the time of infusion until the last visit (12 months post infusion). During the UCBC infusion patients will be monitored at 5 minute intervals, then during hydration every 30 minutes. On leaving the hospital, the patients and their families will be provided with contact details and

asked to contact the study team if any unusual health change should occur. They will also be provided with a card to provide to a treating doctor should the participant require an unscheduled visit, particularly other than at the study site hospital. The card provides details for either the family or the admitting doctor to notify study doctors of potential SAEs to ensure information is elicited, and reported, as early as possible.

The transplant physician will conduct a medical assessment on the day after infusion to assess late reactions to infusion (eg. fever, signs of renal insufficiency), and each week (to detect signs of GvHD) until the 1 month follow up physical examination. At each study visit patients will be asked how they feel and how they have felt in the period since the last visit. They will also be asked if they have had any accidents, illnesses, been hospitalised or changed medication. AEs will also be documented from findings of physical examination.

Assessment and Documentation of Adverse Events
AEs will be reported to the investigator by the infusion team, family or assessing paediatrician or therapist. The investigator is then responsible for recording all AEs, regardless of their association with the study.

The documentation of each AE will include a description of the AE and its severity, timeline, action or treatment required and the outcome. After discussion with the study team, the likelihood that the AE was the result of the study intervention will be assessed as unrelated, possible, probable or definite.

All AEs will be followed until resolved, and changes in the AE or repeated episodes will be documented for retention and publication.

Serious Adverse Event Reporting
Should an AE be categorised as serious, the local HREC will be notified within 72 hours of its occurrence using Appendix 12: Serious adverse event report form. The HREC safety reporting form will be completed, signed and submitted by the coordinating principal investigator with the trial coordinator.
Should there be a SUSAR, HREC will be notified within 72 hours as with a SAE. All SUSARs occurring in a study participant will be reported to the Therapeutic Goods Administration (TGA) within 2 weeks of first occurrence. The report will be completed, signed and submitted by the coordinating principal investigator with the trial coordinator.

Graft-versus-Host Disease (GvHD) of Grade II or above (as defined in Appendix 13:) will stop the trial. The DSMC may advise stopping the trial if other SAEs occur, based on clinical discussion of the SAE and its potential relationship to the study treatment.

9. STATISTICAL METHODS

As the primary aim of this study is to assess safety, the sample size of 12 participants was selected to allow sequential groups of three participants, followed by another three participants, followed by the final six participants. Although we do not expect the cell content of cord blood units to affect safety, and hence a dose escalation safety structure is not required, cell content may relate to the efficacy of the infusion and will be analysed at the conclusion of the study.

While we do not expect the participants to be representative of the general population with CP, we will compare group characteristics with population data from the Australian Cerebral Palsy Register to assess the generalisability of the results obtained. Given the pilot nature of this trial the results from this study will be presented descriptively only. Safety data will be summarised as the proportion of participants who have an SAE and an AE within either of the three safety time periods: within 36 hours, within three months or within the 12 month study period. The change in lab results at each time point will be presented relative to baseline. We will summarise the feasibility data through a summary of the number and proportion of patients recruited of those who were contacted the study team, and a breakdown of the reasons for non-participation. The number of withdrawals and reasons for withdrawal and the number of protocol violations and deviations will also be presented. Change in motor and cognitive function will be presented relative to baseline. Donor cell longevity data will only be presented for the participants receiving sibling UCBC infusions, and categorised as ‘immediate rejection’ to indicate return to baseline fraction of foreign cell-free

DNA within 24 hours; 'rejection' to indicate return to baseline fraction of foreign cell-free DNA by 1 month; 'slow rejection' to indicate the presence of between 200 donor genome equivalent/ml and engraftment at 3 months, and 'engraftment'.

10. DATA MANAGEMENT AND ADMINISTRATIVE ASPECTS

Data collected as part of this trial will be entered into case report forms and a study-specific database, using the internet-based Research Electronic Data Capture (REDCap) database designed specifically for this study which will be accessible only to study staff. While data entry at each site includes participant identification, access for data viewing and analysis will be restricted to re-identifiable format (users will be assigned to groups to restrict access to identification records from other sites). However, all PIs, the trial coordinator and HREC/governance auditors will have access to all records.

To best fulfil regulatory requirements, consent from participant families will be requested in order to report a specific data set to a transplant registry (the Australasian Bone Marrow Transplant Recipient Registry, ABMTRR, and/or the Center for International Blood and Marrow Transplant Research, CIBMTR). The data includes CBU quality information such as TNC and CD34+ count, whether it was washed, how it was administered, and details of any other complication or adverse reaction. After initial data collection, relevant data is followed up at 3 months. This data will be collected as part of the study, but requires specific Optional Consent from the participant family before forwarding to a registry.

While all procedures in this protocol are for research purposes rather than routine clinical care, most procedures will nevertheless be recorded in a participant's medical record. This includes blood type; information about the infusion including observations before and after, and any reaction to the infusion. Information collected as part of this study that will not be recorded in a participant's medical record include CP assessment, study assessments and some blood test results, which will be recorded directly into data collection sheets (case report forms, CRFs). Any medically relevant information or adverse event data collected during these examinations or at other times however will be recorded in the medical record if appropriate. Each participant will be allocated a unique study identification number.

Study samples fall into three categories: 1) samples sent to Red Cross Transplantation Service, will remain fully identifiable and un-coded. 2) Samples collected at infusion hospitals that stay within the hospital and will remain identifiable as well as coded and will be destroyed after use. 3) Samples collected for purely scientific purposes before and after UCBC infusion will be coded and re-identifiable for processing, analysis and storage. As a consequence, many pathology samples will remain identifiable because of the absolute importance that a participant is provided with the correct CBU for infusion and that pathology safety assays are reported and acted upon immediately. Staff will be checking and rechecking identifying details to match with pathology reports.

Video images will be digitised and stored with other electronic records in a password protected folder at each site**.**

All paper and electronic records will be securely stored until the youngest participant turns 25 years of age. Records of biobanked samples and their consent conditions may be retained longer.

Biobank

Samples from each participant, at each time-point, will be stored for future research into the effect of cord blood cells or stem cells on children with neurological disorders. This is important because so much research is being conducted in the area at present that we expect specific research questions to arise in coming years as understanding increases.

Due to its importance, extended consent for storage of samples will not be optional, but will be included in consent for the research study. However, participants and their families will be able to withdraw the participant's samples and information at any time. If this occurs, any remaining samples will be destroyed

and information will no longer be available for future study, however information already included in analysis will not be removed from analysis, just de-identified.

The custodians for this biobank will be the coordinating principal investigator Dinah Reddihough and Associate Investigator Ngaire Elwood in consultation with the trial Steering Committee.

Samples will be collected from all sites and sent to MCRI Biobanking for storage. They may therefore be transported interstate. Samples will at no time leave Australia; any future research will need to be conducted within Australia.

Participant reimbursement

Participants will be reimbursed incurred costs related to this trial. This includes reasonable travel, accommodation and meals expenses, and parking costs. All trial treatments are provided free of charge.

Financial disclosure and conflicts of interest

Cell Care Australia is a private cord blood bank that stands to benefit from a successful outcome of this trial. However, they do not stand to benefit any more than other private cord blood banks that are not associated with this trial, rather, the entire industry should benefit equally.

Cell Care may benefit from publicity associated with the trial. This is affected by the size and scope of the trial, which is decided by the steering committee of which AI Mark Kirkland is a member. There is therefore a potential conflict of interest which has been declared to steering committee and is well recognised.

Use of data and publications policy

Only de-identified data will be reported in any publications or conference presentations. No participant will be identifiable from data reported.

Investigators will submit results for publication regardless of the outcome. Authorship will be determined according to NHMRC guidelines. The decision of what to publish and when, along with authorship, will be made by steering committee. Data will only be published from all sites combined rather than individually, and only at the conclusion of the trial.

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Appendices

Appendix 1: Information to go into CP register newsletters

Australia's first clinical trial of stem cell infusion as a treatment for cerebral palsy is commencing in XXX. The trial is small and aims to find out whether cord blood stem cell infusion is safe for children with cerebral palsy. Unfortunately the trial will not be able to tell us whether stem cell therapy improves function for children with cerebral palsy or not, but it will tell us what the next step should be in this important area in the future.

The clinical trial team are looking for children with cerebral palsy aged 1-16 years who have a brother or sister who has cord blood in storage from their birth. Only around a quarter of brothers and sisters have blood that matches, so the doctors in the trial will test the children's blood to look for a match. Those that are a good match may be able to receive the cord blood cells, but those that do not have a good match cannot as it would not be safe. Involvement in the trial will be demanding with many hospital visits over a 14 month period.

We are looking for about 12 children to be in the trial, across Australia.

If you would like more information about this trial please contact XXX.

If you would like more information about stem cell therapy for cerebral palsy generally, have a look at these websites:

http://www.closerlookatstemcells.org/

http://www.stemcellsaustralia.edu.au/

Appendix 2: Script for first contact phone conversation (families contacting research team)

Hi, my name is [insert researcher name]. Have you heard about the clinical trial of umbilical cord blood cell therapy? Would you like me to tell you a little about the trial, or do you have any questions already?

[pause to answer questions, describe the study including but not limited to the below]

This trial is using the stem cells, from a brother or sister's cord blood but only if they match, and the chance of matching is about 25%. Therefore, this trial can only include children with CP who have a siblings' cord blood in storage somewhere. Since only 25%, a quarter, of siblings have matching cord blood, the trial will involve testing sibling cord blood to see if it matches and can be used, or if it can't be used. Three quarters won't match. We expect that only a small group will be eligible for this trial.

Altogether, we hope to find 12 children from across Australia, who are 1-16 years old, who can participate in this trial. It doesn't matter where the families live in Australia, but families that don't live in Brisbane or Melbourne would have to cope with quite a lot of travel. The trial may be able to provide some help to cover the costs of the travel.

We don't know whether or not there are 12 children in Australia who are eligible to participate, whose families will choose to enrol them in this trial, and who we find have matching cord blood when we test it. There may not be 12 children like this in Australia, or there may be lots of children like this. Even if there are more than 12 children like this in Australia, we can only have 12 participate.

There is a list of other things that make the children eligible. Some of them are fairly clear:

- Being at least 1 year old at the time of enrolment (so they don't have to be 1 year old just yet)
- Being less than 17 years old at the time of enrolment
- Having cerebral palsy, not any other disorder
- Not having an immune system disorder or immune deficiency
- Not needing ventilator support
- Being able to travel to the hospital
- Not having any other cell therapy

Some of the things are a little less clear, because they may change, for example if a child is unwell they won't be able to have the infusion, but if it is a virus the doctor may say that your child will get better in time to participate. Also, if a child has a genetic disorder or brain dysplasia the cord blood infusion probably won't help at all, but only some people have had genetic testing or MRI done. [Do not mention exclusion periods for Botulinum toxin A or surgery at this point].

Would you mind if I ask a couple of questions to find out if your child might be eligible for this trial?

- Does your child have CP?
- Who diagnosed your child's CP? For example, was it a doctor?

[if child is clearly ineligible]
It sounds like your child would not be able to participate in this trial, because this trial only includes children who have CP.

- Do you happen to remember if you donated or stored cord blood at the births of any of your children?

[if they didn't]

Well, unfortunately that rules this trial out I'm afraid. I don't know what will happen in the future, some researchers are looking at whether cord blood cells from an unrelated donor could be helpful for children with cerebral palsy. It's possible that after this small pilot trial there may be an Australian trial of unrelated donor cord blood, but it is a long way off. Keep listening out over the next few years, any clinical trials will be publicised in the same ways that this one is.

[if they did]
Oh fantastic. Was that for your child who has CP, or their brother or sister?

Do you know if it was a donation to a public (AusCord) cord blood bank in case someone with leukaemia or other problems needed it, or did you pay for the storage with a private service?

[if through AusCord]
We can't include cord blood units that are stored in the public banks at the moment. We hope that this will change, and if you would like I can take your details and contact you if it does change.

The three public banks keep the donations separate, but they all work together as AusCord. The cord blood that is donated to AusCord gets used all over the world to help people who are sick with blood and marrow disorders and metabolic disorders. Sometimes it also gets used for research. The cord blood you donated might still be stored here, or it may have been used. If the trial is allowed to include cord blood stored in public banks, we will ask you some questions to find out which hospital and state you gave birth in, but I can contact you then.

[if through a private bank]
Ok, that should be easy to find then.

We can find out the status of your cord blood unit by giving the cord blood bank some details to do a search. Would you like to do that? They need to know your child's date of birth, hospital of birth, sex and mother's details. I will post you a template letter for you to fill out with your information, sign, and send back to me if you are happy for us to find out and for the cord blood bank to tell us that they have your cord blood on record. This doesn't sign your child up for the clinical trial; it is only for finding out about the cord blood. It is important to know that there may be other reasons why your child cannot participate in the trial, from the eligibility criteria we discussed earlier.

Also, please remember that sibling cord blood might match or it might not. Only around a quarter of siblings match, so that means that three quarters of children with sibling cord blood available will not be able to use the sibling cells. Also, I need to ask: Does your child have an immune system disorder, or an immune deficiency? If your child has an immune system disorder or an immune deficiency, having someone else's cells in your child's body is less safe.

[if the child might have an immune system disorder or immune deficiency]
It sounds like your child would not be able to participate in this trial, because if your child has an immune system disorder or an immune deficiency, infusing someone else's cells into your child's body using this study's protocol is less safe

[all]
I've jotted down some details as we've talked. May I include the details you have been telling me in our database? I will send you a letter with a list of what we've talked about so that you know what we have recorded and how to contact us to change anything, ask for your information to be removed or to ask questions. Could I please get your postal address so that I can mail it to you (along with the template letter for the cord blood banks)?

Now, if we find that your cord blood donation is still in storage, or if you know it is in a private bank, and if you are still interested in the trial, I will also send you a full Information Statement about the whole trial. The

Information Statement gives information about reasons your child might not be able to participate in the trial, which is important because we do not want to infuse any child when it isn't safe. The Information Statement also has all the details and risks written down in it for you to think about. You can talk about it with your child's doctor.

Look out for a letter in the mail from us in the next few days, it will list what we talked about today, and also includes the template letter for you to fill in and send back to us with a reply-paid envelope that will be included [and/or] an Information Statement for you to think about. Our phone number will be on the letter and please call back anytime with more questions or to talk about it more. There are lots of things to think about with this trial and it is a very personal decision for each family.

Thanks for your time; I look forward to talking again soon.

Appendix 3: Record of verbal consent

RECORD OF VERBAL CONSENT

HREC Project Number: HREC/14/RCHM/38 RCH HREC ID: 34210

Research Project Title: **Safety study of sibling cord blood cell infusion to children with cerebral palsy**

Participant Name:

Parent Name:
Mailing Address:
Phone Number:

Date that family contacted research team:
Date of actual phone conversation:
Spoke to (name of parent/participant):

☐ Yes	☐ No	Do you give your consent for me to ask some questions about your child? I would like to find out if they are suitable for this research project.
☐ Yes	☐ No	Do you give your consent to store the information you gave me in the study database?

Name of person obtaining consent:
Signature of Person Obtaining Consent:

Date:	Time:

Appendix 4: Letter following verbal consent

<Date>

<Address block>
<Address block>
<Address block>

Dear <parent name>,

I would like to thank you and your child for your interest in research into umbilical cord blood cells for treatment of children with cerebral palsy.

I have recorded that you gave verbal consent for us to store the information you gave us over the phone in the study database.

We recorded x pieces of information. They are:

1. That your child has cerebral palsy
2. Whether you stored cord blood for any of your children at their births
3. If so, which cord blood bank you stored it with
4. Your contact details

You are welcome to change your mind at any time without giving a reason, and withdraw your consent for storing this information. If you do, we will not store or use the above information in the future. If you have questions, want to find out more about the information you gave us, or to change or update the information, please contact XXX on <phone number>.

You will also find in this envelope [either] a template letter for filling in and signing along with a prepaid envelope for sending it back to us if you want us to investigate your cord blood unit(s) [and/or] the Study Information Statement.

Thanks so much for your time and for supporting research into cerebral palsy.

With kind regards,

Clinical Trial Coordinator
Contact details

Appendix 5: Template to ask CBB to locate CBU records

Does my child/do my children have their cord blood in storage?

Using the information below, could you please ask the cord blood banks to locate the records of my child / children's cord blood unit? These are details from the children who may have stored or donated cord blood.

	Child 1	Child 2
Child's name at birth		
Child's Date of birth		
Hospital where child was born		
Mother's name when the child was born		
Which cord blood bank did you use?		

Mother's date of birth: _____

I would like to know the results of the record search.
I give permission for the cord blood bank to release information to the research team and for the research team to release information to me.

Name of child with cerebral palsy: _____

Parent/Guardian name: _____

Parent/Guardian signature: _____

Date: _____

Appendix 6: Script for phone conversation after CBU record search

Hello, this is [lead site study coordinator's name], is this a good time to call or would you prefer me to call back later?

The cord blood bank has looked through their records for the cord blood that you remember having collected when your child was born.

[Either]
They found that the cord blood is not in storage anymore, it was used to help someone who was sick / was used for research to improve cord blood banking and cord blood use / was contaminated / they have no record of it. This means that your child will not be able to participate in this trial. Would you like to be contacted if a future clinical trial might be appropriate for your child? We don't know if this will happen, but we are keeping a list of families interested in this research.

[if family requested a record search in a public bank]
We can't include cord blood units that are stored in the public banks at the moment. We hope that this will change, and if you would like I can take your details and contact you if it does change.

Even if we are allowed to include cord blood units stored in the public banks in the future, getting access to your child's cord blood is difficult because your child's cord blood may be useful for someone sick. The cord blood bank may be able to release it if you apply for it. It will take some time or the bank to process your application. They would like to support this research, but the bank's first priority is people who will definitely benefit from the cord blood cells – and in this trial we do not know if your child will receive any benefit at all.

[or]

They found that the cord blood is in storage.

[for all]
If you are interested in finding out more about this research study, I can mail you a copy of the Information Statement for you to have, so that you can think about it. It would be a great idea to talk to your child's doctor about it, talk within your family and spend some time thinking about it. You may decide that this study isn't something you want your child involved in, which is fine, or that your child might not be eligible to participate for other reasons listed in the Information Statement. We could discuss this now if it would help too? [Explain eligibility criteria if appropriate, see previous script].

Now that you know that there is a cord blood unit in storage from one of your children, something to think about when you read the Information Statement is that this trial may use the cord blood up. The cord blood would not be there in case any of your family happened to get sick and need the cord blood. I truly hope that wouldn't happen, but it is something your whole family need to think about.

[Do not raise issue of trial possibly being oversubscribed at this point, unless family ask].

You already know this phone number, so please call anytime with questions. Also, contact details for the study team are on the Information Statement.

Please take a few weeks to think about it and talk it through with others, and if you think you would like your child to participate in this research study, and that your child fits the other eligibility criteria, particularly that your child does not have an immune disorder [explain criteria again if appropriate], we can arrange a time for you to come to a study hospital and talk with the coordinator there (it might be me), or with a study doctor.

Also, if you are thinking of enrolling your child in the study, before you come in we would like to know a little bit more about your child's cerebral palsy. We could look at a letter from your child's doctor, or some other

documentation, that has your child's diagnosis in it. Of if you don't have something like that, we would ask your permission to contact your child's doctor. But don't worry too much about that yet, spend some time thinking about this study first.

I will call you again in a few weeks if we haven't heard from you, just to find out if the Information Statement makes sense and if you have any questions. But we don't expect you to have thought about everything by that point, or made any decisions don't worry.

Thank you for your time.

Appendix 7: Script for phone conversation to arrange Visit 1

Hello, this is [lead site study coordinator's name], how can I help you, do you have any questions about the research study?

Oh, hi [parent name], you are interested in coming in to talk about the study and possibly enroll [child's name]? Ok. First, would you mind if I just run through the list of eligibility criteria one more time, like a checklist?

1) How old is your child?
2) Has your child got cerebral palsy? Has your child ever had genetic testing done either to try to explain the cerebral palsy or for any other reason?
3) Has your child ever had a brain scan called a MRI? Do you happen to know what it showed?
4) Has your child's neurological condition or motor functioning gotten progressively worse over time?
5) Has your child ever had cell therapy before?
6) We need to know if your child has ever been diagnosed with an immune system disorder or with an immune deficiency? If you are not sure don't worry, we will test for it anyway because we don't want to give an infusion if it is more risky than normal.
7) Is your child on a ventilator?
8) Will your child and you or another parent or guardian able to travel to a study hospital?
9) Is your child likely to need Botulinum toxin A (Botox) treatment in the next year or so? When would you expect it to happen?
10) Is your child likely to need any sort of surgery in the next year or so? Any idea what sort of surgery, or when it would be?

[Either]
From your answers, your child will not be able to participate in this study. It would not be safe for your child / this study isn't appropriate but perhaps a future study might be.

[Or]
From your answers, I don't know if your child will be able to participate or not. It may be something that you need to talk about with the study doctor. Would you prefer to speak to the study doctor over the phone, or to come to the hospital and talk in person? It is really important that we don't make mistakes about this.

Let's try to find a time for you to come to the hospital. We can't arrange a meeting for [xxx] weeks because we don't want you to go to the trouble of coming to the hospital if the trial is full, and we don't know if it is full right this minute because we are waiting on results from children that enrolled recently.

When you and your child come to the hospital, you will meet the trial coordinator (which might be me), and maybe the study doctor. We can help answer any more questions and discuss the risks and how much effort participating in the study might involve for your family.

Then, if you choose, you can enroll your child by signing a Consent Form, but you may choose not to enroll on this day, or at all.

If you do choose to enroll your child, your child will then go to the hospital pathology collection service to give a blood sample. This sample would be sent to the Australian Red Cross Blood Service Transplant Service to be tested for tissue type. If the blood bank does not know the tissue type of the cord blood unit in storage, a small amount of cord blood will be sent as well to find out whether it matches. A cord blood transplant specialist will receive the results but will not tell us or your family what the results are unless the study has already got enough participants. Because children enroll before the transplant specialist knows whether their cord blood can be used or not, it is difficult to tell you whether the trial will be full or not.

In the meantime, we would like to know a little bit more about your child's cerebral palsy, to make sure your child is eligible before you go to the effort of coming to the hospital. Do you have a copy of a letter from your child's doctor, or some other documentation, that has your child's diagnosis in it? Do you think it would be possible to mail us a copy, I will send out a letter to you describing what is needed with a reply-paid envelope for you to mail the document back to us. If you can't find a letter from your child's doctor, don't worry, we would ask your permission to contact your child's doctor instead.

Please call any time to talk about any part of the study, and look out in the mail for our letter. Thank you for your time.

Appendix 8: Letter to family to request confirmation of diagnosis

<Date>

<Address block>
<Address block>
<Address block>

Dear <parent name>,

I would like to thank you and your child for your interest in research into umbilical cord blood cells for treatment of children with cerebral palsy.

Before you visit a study hospital, we would like to know more about your child's cerebral palsy. Please fill in the attached form. If you have a letter from your child's doctor that says your child's diagnosis, or some other document with your child's diagnosis, please send a copy with the form to the study team using the reply-paid envelope included.

If you don't have a letter or document available, the study doctor could contact your child's doctor with your permission. The study doctor would send a copy of the attached form to show your child's doctor that you give permission to discuss your child's cerebral palsy. The study doctor would tell your child's doctor about the research study, that you have not enrolled your child at this time, and why we would like the information.

If you would like to talk about this, or ask any other questions, please call anytime.

Thank you,

Clinical Trial Coordinator
Contact details

Form requesting confirmation of diagnosis

Name of child: _____

Name of child's doctor: _____

Contact details for child's doctor:

Address: _____

_____

_____

Phone: _____

☐ I have ☐ I have not attached a letter from my child's doctor that includes my child's diagnosis.

☐ I do ☐ I do not give my permission for the study team to contact my doctor to get confirmation of my child's diagnosis

Comments: _____

_____

_____

_____

Name of Parent / Guardian: _____

Signature of Parent / Guardian: _____

Appendix 9: Letter to child's doctor requesting information

<Date>

<Address block>
<Address block>
<Address block>

Dear <doctor's name>,

Regarding: <child's name>.

We are seeking evidence of cerebral palsy diagnosis for <child's name>, and have permission from <parent/guardian name> to contact you for this information. Please see attached written consent.

The information will be used to consider one aspect of eligibility of <child's name> for enrolment in a clinical trial of single dose intravenous cord blood cell infusion, combined with intensive rehabilitation therapy, as a possible treatment for cerebral palsy, called '

Safety study of sibling cord blood cell infusion to children with cerebral palsy'.

The trial is a safety study, conducted by an Australian collaborative research group at The Royal Children's Hospital Melbourne and the Lady Cilento Children's Hospital, Brisbane (Coordinating Principal Investigator Dr Dinah Reddihough). This project has received ethics approval from The Royal Children's Hospital Human Research Ethics Committee (Project number HREC/14/RCHM/38; RCH HREC ID: 34210).

Before we can consider further including <child's name> in the study, we need to be sure that he / she does in fact have cerebral palsy. We would very much appreciate a brief note confirming this.

If you would like to discuss this with the study doctor at <likely infusion hospital>, please call <study doctor name and contact>.
If you would like more information about the clinical trial, please call <study coordinator's name and contact>.
If you would like more information about the ethics approval for this study, please contact <Director, Research Ethics and Governance, contact, of which site>

Thank you for your time,

Study doctor

Contact details

Appendix 10: Screening questions to establish immune status for medical history

- If answer Yes to (3), (4), (5), (7), should undergo immune blood tests before deciding if need to be excluded.
- If participant needs to have immune blood tests AND answers Yes to (1) and/or (8), consider deferring blood test until free from illness for 7 days / free from blood products for 3 months.
- If participant needs to have immune blood tests, please record all relevant history (i.e. to which question did they answer Yes) in the clinical background section of the pathology request slip.

Y / N

1) Has your child had an infection or a febrile (high temperature) illness within the last seven (7) days? ☐

If Yes, and if sibling CBU in storage, consider deferring visit 2 blood test until free from illness for 7 days

2) Has your child ever been diagnosed with an immune system disorder or immune deficiency syndrome? ☐

If yes(Y), please specify:

If Yes, exclude from participation

3) Has your child needed to be admitted to hospital for intravenous (I.V.) antibiotics to clear an infection on more than two (2) occasions in their life? ☐

If Yes, consult with immunologist regarding blood test results before deciding to exclude

4) Has your child ever needed two (2) or more months of oral antibiotics to clear an infection? ☐

If Yes, consult with immunologist regarding blood test results before deciding to exclude

5) Has any Doctor or Maternal Child Health Nurse ever been concerned about your child being underweight? ☐

If Yes, consult with immunologist regarding blood test results before deciding to exclude

6) Has your child ever been diagnosed with a genetic disorder such as Down syndrome, Di George syndrome etc? ☐

If yes(Y), please specify:

If Yes, exclude from participation

7) Has any family member or relative of your child ever been diagnosed with an immune system disorder or immune deficiency syndrome? ☐

If yes(Y), please specify:

If Yes, consult with immunologist regarding blood test results before deciding to exclude

8) Has your child received any blood products such as packed red blood cells, platelets, albumin or immunoglobulin in the last three (3) months? ☐

If yes(Y), please specify:

If Yes, and if sibling CBU in storage, consider deferring visit 2 blood test until free from blood products for 3 months

9) Was your child born prematurely, before 36 weeks' gestation? ☐

Appendix 11: Post-infusion handout

Title	*Safety study of sibling cord blood cell infusion to children with cerebral palsy*
Short Title	*Stem Cells in Umbilical Blood Infusion for Cerebral Palsy (SCUBI-CP)*
HREC Number	*HREC/14/RCHM/38*
Principal Investigator	*Site PI*
Location	*Site*

Post-infusion handout

Your child will not be released from the hospital unless he/she is healthy enough to leave. In particular, the nurse will check

- your child's blood pressure
- whether your child is urinating freely
- whether there is haemoglobin in your child's urine.

We would like to check your child again tomorrow. The doctor will look for signs that your child is having a delayed reaction to the infusion.

The following list of symptoms might be linked to the cord blood cell infusion:

Possible symptoms	**What it may mean**	**When it might happen**
Your child's urine is purple	There may be haemoglobin in your child's urine.	This is most likely within 36 hours of infusion.
Nausea or diarrhoea	Your child may be reacting to the infused cells	This is most likely between 1 week and 3 months after infusion
Jaundice (yellowing of your child's skin)	Your child may be reacting to the infused cells	This is most likely between 1 week and 3 months after infusion
Fever	Your child may have an infection	This is most likely within 36 hours of infusion
Rash	Your child may be reacting to the infused cells	This is most likely between 1 week and 3 months after infusion

If you notice that your child has any of these symptoms, please call the 24 hour phone line to talk to a transplant doctor:

Call: ***(local 24 hour contact number)***
Ask for: **BMT transplant doctor**
Tell the doctor: Your child has cerebral palsy and is in the trial

If you feel your child has serious health problems of any type, please bring them to the Emergency Department at *site*, or dial **000**.

Appendix 12: Serious adverse event report form

SAE REPORT FORM (Individual Reports)

Internal* SAEs (occurring to RCH participants) **must be reported to the RCH HREC within 24-72 hours of occurrence and must be accompanied by a detailed report of the event.**
External SAEs (occurring to participants from other sites) **must be reported in a prompt manner if the information impacts the continued ethical acceptability of the trial or requires documentation to be updated (i.e. protocol or PIS).**
***An Internal SAE is one occurring in a participant that RCH researchers are responsible for, this is independent of where the event occurs.**

HREC Reference #	
Project title	
Principal Investigator	

Date that SAE occurred :	
Date Investigator became aware of SAE :	
Participant ID :	
Internal or External (see above definition):	
Event description and management :	
Event outcome (synopsis):	
Expectedness of the SAE (PI Opinion):	☐ Expected ☐ Unexpected
Relationship to the study drug	☐ Unrelated ☐ Possibly related ☐ Probably Related ☐ Definitely Related

Other Comments	
Does the protocol require amending as a result of these SAEs? (If Yes, please submit a modification request with the amended protocol)	☐ Yes ☐ No
Do the information statements require amending as a result of these SAEs? (If Yes, please submit a modification request with the amended forms)	☐ Yes ☐ No

Signature	Date

Please submit 1 hard copy with signature OR an electronic copy signed (or emailed) by the PI to rch.ethics@rch.org.au

Appendix 13: Categorising severity of AEs related to study treatments: NCI Common Terminology Criteria for AEs (CTCAE)

Note that AEs not considered to be related to study treatments are not included here. Additionally, note that definitions including self-care and activities of daily living (ADL) must be considered relative to the child's previous level of functional impairment.

Adverse Event		**Grade**				
	When it may occur	**1**	**2**	**3**	**4**	**5**
Blood disorders						
Haemolysis Definition: A disorder characterized by laboratory test results that indicate widespread erythrocyte cell membrane destruction.	• Within 24 hours after infusion • 2-14 days after infusion	Laboratory evidence of haemolysis only (e.g., direct antiglobulin test; DAT; Coombs; schistocytes; decreased haptoglobin)	Evidence of haemolysis and >=2 gm decrease in haemoglobin.	Transfusion or medical intervention indicated (e.g., steroids)	Life-threatening consequences; urgent intervention indicated	Death
Thrombotic thrombocytopenic Purpura Definition: A disorder characterized by the presence of microangiopathic haemolytic anaemia, thrombocytopenic purpura, fever, renal abnormalities and neurological abnormalities such as seizures, hemiplegia, and visual disturbances. It is an acute or subacute condition.	• 5-12 days after infusion	Evidence of RBC destruction (schistocytosis) without clinical consequences	-	Laboratory findings with clinical consequences (e.g., renal insufficiency, petechiae)	Life-threatening consequences, (e.g., CNS haemorrhage or thrombosis/embolism or renal failure)	Death
Cardiac disorders						
Heart failure Definition: A disorder characterized by the inability of the heart to pump blood at an adequate volume to meet tissue metabolic requirements, or, the ability to do so only at an elevation in the filling pressure.	•	Asymptomatic with laboratory (e.g., BNP [B-Natriuretic Peptide]) or cardiac imaging abnormalities	Symptoms with mild to moderate activity or exertion Severe with symptoms at rest or	Severe with symptoms at rest or with minimal activity or exertion; intervention indicated	Life-threatening consequences; urgent intervention indicated (e.g., continuous IV therapy or mechanical haemodynamic support)	Death
Sinus bradycardia Definition: A disorder characterized by a dysrhythmia with a heart rate less than 60 beats per minute that originates in the sinus node.	• During or immediately after infusion	Asymptomatic, intervention not indicated	Symptomatic, medical intervention indicated	Severe, medically significant, medical intervention indicated	Life-threatening consequences; urgent intervention indicated	Death

Sinus tachycardia A disorder characterized by a dysrhythmia with a heart rate greater than 100 beats per minute that originates in the sinus node.	• During or immediately after infusion	Asymptomatic, intervention not indicated	Symptomatic; non-urgent medical intervention indicated	Urgent medical intervention indicated	-	-
Supraventricular tachycardia Definition: A disorder characterized by a dysrhythmia with a heart rate greater than 100 beats per minute that originates above the ventricles.	• During or immediately after infusion	Asymptomatic, intervention not indicated	Non-urgent medical intervention indicated	Medical intervention indicated	Life-threatening consequences; urgent intervention indicated	Death
Ventricular arrhythmia Definition: A disorder characterized by a dysrhythmia that originates in the ventricles.	• During or immediately after infusion	Asymptomatic, intervention not indicated	Non-urgent medical intervention indicated	Medical intervention indicated	Life-threatening consequences; hemodynamic compromise; urgent intervention indicated	Death
Ventricular tachycardia Definition: A disorder characterized by a dysrhythmia with a heart rate greater than 100 beats per minute that originates distal to the bundle of His.	• During or immediately after infusion	-	Non-urgent medical intervention indicated	Medical intervention indicated	Life-threatening consequences; hemodynamic compromise; urgent intervention indicated	Death
Gastrointestinal disorders						
Diarrhoea	• Diarrhoea includes diarrhoea of small bowel or colonic origin, and/or ostomy diarrhoea.	Increase of <4 stools per day over baseline; mild increase in ostomy output compared to baseline	Increase of 4 – 6 stools per day over baseline; IV fluids indicated <24hrs; moderate increase in ostomy output compared to baseline; not interfering with ADL	Increase of ≥7 stools per day over baseline; incontinence; IV fluids ≥ 24 hrs; hospitalization; severe increase in ostomy output compared to baseline; interfering with ADL	Life-threatening consequences (e.g., hemodynamic collapse)	Death
Mucositis / stomatitis	• Mucositis/stomatitis (functional/symptomatic) may be used for identifying GvHD.	Erythema of the mucosa	Patchy ulcerations or pseudo-membranes	Confluent ulcerations or pseudo-membranes; bleeding with minor trauma	Tissue necrosis; significant spontaneous bleeding; life-threatening consequences	Death

Nausea Definition: A disorder characterized by a queasy sensation and/or the urge to vomit.	• During or immediately after infusion • May be early indication of allergic reaction or anaphylaxis	Loss of appetite without alteration in eating habits	Oral intake decreased without significant weight loss, dehydration or malnutrition	Inadequate oral caloric or fluid intake; tube feeding, TPN, or hospitalization indicated	-	-
Vomiting Definition: A disorder characterized by the reflexive act of ejecting the contents of the stomach through the mouth.	• During or immediately after infusion • May be early indication of allergic reaction or anaphylaxis	1 - 2 episodes (separated by 5 minutes) in 24 hrs	3 - 5 episodes (separated by 5 minutes) in 24 hrs	≥6 episodes (separated by 5 minutes) in 24 hrs; tube feeding, TPN or hospitalization indicated	Life-threatening consequences; urgent intervention indicated	Death
General disorders and administration site conditions						
Chills Definition: A disorder characterized by a sensation of cold that often marks a physiologic response to sweating after a fever.	• During or immediately after infusion • Within 36 hours of infusion	Mild sensation of cold; shivering; chattering of teeth	Moderate tremor of the entire body; narcotics indicated	Severe or prolonged, not responsive to narcotics	-	-
Fever Definition: A disorder characterized by elevation of the body's temperature above the upper limit of normal.	• During or immediately after infusion • Within 36 hours of infusion	38.0 - 39.0 ºC	>39.0 - 40.0 ºC	>40.0 ºC	>40.0 ºC for >24 hrs	Death
Headache Definition: A disorder characterized by a sensation of marked discomfort in various parts of the head, not confined to the area of distribution of any nerve.	• During or immediately after infusion • Up to 36 hours after infusion	Mild pain	Moderate pain; limiting instrumental ADL	Severe pain; limiting self-care ADL	-	-
Infusion related reaction Definition: A disorder characterized by adverse reaction to the infusion of pharmacological or biological substances.	• During or immediately after infusion	Mild transient reaction; infusion interruption not indicated; intervention not indicated	Therapy or infusion interruption indicated but responds promptly to symptomatic treatment (e.g., antihistamines, NSAIDS, narcotics, IV fluids); prophylactic medications indicated for ≤24hrs	Prolonged (e.g., not rapidly responsive to symptomatic medication and/or brief interruption of infusion); recurrence of symptoms following initial improvement; hospitalization indicated for clinical sequelae	Life-threatening consequences; urgent intervention indicated	Death

Infusion site extravasation Definition: A disorder characterized by leakage of a pharmacologic or a biologic substance from the infusion site into the surrounding tissue. Signs and symptoms include induration, erythema, swelling, burning sensation and marked discomfort at the infusion site.	• During or immediately after infusion	-	Erythema with associated symptoms (e.g., edema, pain, induration, phlebitis)	Ulceration or necrosis; severe tissue damage; operative intervention indicated	Life-threatening consequences; urgent intervention indicated	Death
Injection site reaction Definition: A disorder characterized by an intense adverse reaction (usually immunologic) developing at the site of an injection.	• During or immediately after infusion	Tenderness with or without associated symptoms (e.g., warmth, erythema, itching)	Pain; lipodystrophy; oedema; phlebitis	Ulceration or necrosis; severe tissue damage; operative intervention indicated	Life-threatening consequences; urgent intervention indicated	Death
Irritability Definition: A disorder characterized by an abnormal responsiveness to stimuli or physiological arousal; may be in response to pain, fright, a drug, an emotional situation or a medical condition.	•	Mild; easily consolable	Moderate; limiting instrumental ADL; increased attention indicated	Severe abnormal or excessive response; limiting self-care ADL; inconsolable	-	-
Localized oedema Definition: A disorder characterized by swelling due to excessive fluid accumulation at a specific anatomic site.	• Within 24 hours of infusion • Pulmonary oedema may be indication of transfusion-associated circulatory overload (TACO)	Localized to dependent areas, no increased disability or functional impairment	Moderate localized oedema and intervention indicated; limiting instrumental ADL	Severe localized oedema and intervention indicated; limiting self-care ADL	-	-
Non-cardiac chest pain Definition: A disorder characterized by discomfort in the chest unrelated to a heart disorder.	• During or immediately after infusion	Mild pain	Moderate pain; limiting instrumental ADL	Severe pain; limiting self-care ADL	-	-
Pain Definition: A disorder characterized by the sensation of marked discomfort, distress or agony.	• During or immediately after infusion	Mild pain	Moderate pain; limiting instrumental ADL	Severe pain; limiting self-care ADL	-	-
Immune system						

Allergic reaction Definition: A disorder characterized by an adverse local or general response from exposure to an allergen.	• During or immediately after infusion	Transient flushing or rash, drug fever <38ºC; intervention not indicated	Intervention or infusion interruption indicated; responds promptly to symptomatic treatment (e.g., antihistamines, NSAIDS, narcotics); prophylactic medications indicated for ≤24 hrs	Prolonged (e.g., not rapidly responsive to symptomatic medication and/or brief interruption of infusion); recurrence of symptoms following initial improvement; hospitalization indicated for clinical sequelae (e.g., renal impairment, pulmonary infiltrates)	Life-threatening consequences; urgent intervention indicated	Death
Anaphylaxis Definition: A disorder characterized by an acute inflammatory reaction resulting from the release of histamine and histamine-like substances from mast cells, causing a hypersensitivity immune response. Clinically, it presents with breathing difficulty, dizziness, hypotension, cyanosis and loss of consciousness and may lead to death.	• During or immediately after infusion	-	-	Symptomatic bronchospasm, with or without urticaria; parenteral intervention indicated; allergy-related oedema/angioedema; hypotension	Life-threatening consequences; urgent intervention indicated	Death
Cytokine release syndrome Definition: A disorder characterized by nausea, headache, tachycardia, hypotension, rash, and shortness of breath; it is caused by the release of cytokines from the cells.	•	Mild reaction; infusion interruption not indicated; intervention not indicated	Therapy or infusion interruption indicated but responds promptly to symptomatic treatment (e.g., antihistamines, NSAIDS, narcotics, IV fluids); prophylactic medications indicated for ≤24 hrs	Prolonged (e.g., not rapidly responsive to symptomatic medication and/or brief interruption of infusion); recurrence of symptoms following initial improvement; hospitalization indicated for clinical sequelae (e.g., renal impairment, pulmonary infiltrates)	Life-threatening consequences; pressor or ventilatory support indicated	Death
Infection						
Catheter related infection Definition: A disorder characterized by an infectious	• Within 24 hours of infusion	-	Localized; local intervention indicated; oral	IV antibiotic, antifungal, or antiviral intervention indicated; radiologic or	Life-threatening consequences;	Death

process that arises secondary to catheter use.			intervention indicated (e.g., antibiotic, antifungal, antiviral)	operative intervention indicated	urgent intervention indicated	
Respiratory infection Definition: A disorder characterized by an infectious process involving the respiratory tract.	• Throughout study	-	Moderate symptoms; oral intervention indicated (e.g., antibiotic, antifungal, antiviral)	IV antibiotic, antifungal, or antiviral intervention indicated; radiologic, endoscopic, or operative intervention indicated	Life-threatening consequences; urgent intervention indicated	Death
Procedural complications						
Bruising Definition: A finding of injury of the soft tissues or bone characterized by leakage of blood into surrounding tissues.	• During or immediately after needle	Localized or in a dependent area	Generalized	-	-	-
Venous injury Definition: A finding of damage to a vein.	• During or immediately after needle	Asymptomatic diagnostic finding; intervention not indicated	Symptomatic (e.g., claudication); repair or revision not indicated	Severe symptoms; limiting self-care ADL; repair or revision indicated; disabling	Life-threatening consequences; evidence of end organ damage; urgent operative intervention indicated	Death
Psychiatric disorders						
Agitation Definition: A disorder characterized by a state of restlessness associated with unpleasant feelings of irritability and tension.	• During or immediately after infusion May be early indication of anaphylaxis	Mild mood alteration	Moderate mood alteration	Severe agitation; hospitalization not indicated	Life-threatening consequences; urgent intervention indicated	Death
Anxiety Definition: A disorder characterized by apprehension of danger and dread accompanied by restlessness, tension, tachycardia, and dyspnea unattached to a clearly identifiable stimulus.	• Throughout study	Mild symptoms; intervention not indicated	Moderate symptoms; limiting instrumental ADL	Severe symptoms; limiting self-care ADL; hospitalization not Indicated	Life-threatening; hospitalization indicated	Death
Confusion Definition: A disorder characterized by a lack of clear and orderly thought and behaviour.	•	Mild disorientation	Moderate disorientation; limiting instrumental ADL	Severe disorientation; limiting self-care ADL	Life-threatening consequences; urgent intervention indicated	Death

Depression Definition: A disorder characterized by melancholic feelings of grief or unhappiness.	• Throughout study	Mild depressive symptoms	Moderate depressive symptoms; limiting instrumental ADL	Severe depressive symptoms; limiting self-care ADL; hospitalization not indicated	Life-threatening consequences, threats of harm to self or others; hospitalization indicated	Death
Restlessness Definition: A disorder characterized by an inability to rest, relax or be still.	• During or immediately after infusion May be early indication of anaphylaxis	Mild symptoms; intervention not indicated	Moderate symptoms; limiting instrumental ADL	Severe symptoms; limiting self-care ADL	-	-
Renal and urinary disorders						
Haemoglobinuria Definition: A disorder characterized by laboratory test results that indicate the presence of free haemoglobin in the urine.	• During or immediately after infusion • Within 36 hours of infusion	Asymptomatic; clinical or diagnostic observations only; intervention not indicated	-	-	-	-
Respiratory, thoracic and mediastinal disorders						
Bronchospasm Definition: A disorder characterized by a sudden contraction of the smooth muscles of the bronchial wall.	• During or immediately after infusion	Mild symptoms; intervention not indicated	Symptomatic; medical intervention indicated; limiting instrumental ADL	Limiting self-care ADL; oxygen saturation decreased	Life-threatening respiratory or hemodynamic compromise; intubation or urgent intervention indicated	Death
Dyspnea Definition: A disorder characterized by an uncomfortable sensation of difficulty breathing.	• During or immediately after infusion	Shortness of breath with moderate exertion	Shortness of breath with minimal exertion; limiting instrumental ADL	Shortness of breath at rest; limiting self-care ADL	Life-threatening consequences; urgent intervention indicated	Death
Hypoxia Definition: A disorder characterized by a decrease in the level of oxygen in the body.	• During or immediately after infusion	-	Decreased oxygen saturation with exercise (e.g., pulse oximeter <88%); intermittent supplemental oxygen	Decreased oxygen saturation at rest (e.g., pulse oximeter <88% or PaO2 ≤55 mm Hg)	Life-threatening airway compromise; urgent intervention indicated (e.g., tracheotomy or intubation)	Death
Respiratory failure Definition: A disorder characterized by impaired gas exchange by the respiratory system resulting in hypoxemia and a decrease in oxygenation	• During or immediately after infusion	-	-	-	Life-threatening consequences; urgent intervention, intubation, or ventilatory support indicated	Death

of the tissues that may be associated with an increase in arterial levels of carbon dioxide.						
Stridor Definition: A disorder characterized by a high pitched breathing sound due to laryngeal or upper airway obstruction.	• During or immediately after infusion	-	-	Respiratory distress limiting self-care ADL; medical intervention indicated	Life-threatening airway compromise; urgent intervention indicated (e.g., tracheotomy or intubation)	Death
Wheezing Definition: A disorder characterized by a high-pitched, whistling sound during breathing. It results from the narrowing or obstruction of the respiratory airways.	• During or immediately after infusion	Detectable airway noise with minimal symptoms	Moderate symptoms; medical intervention indicated; limiting instrumental ADL	Severe respiratory symptoms limiting self-care ADL; oxygen therapy or hospitalization indicated	Life-threatening consequences; urgent intervention indicated	Death
Skin and subcutaneous tissue disorders						
Rash/desquamation	• Rash/desquamation may be used for identifying GvHD	Macular or popular eruption or erythema without associated symptoms	Macular or popular eruption or erythema with pruritus or other associated symptoms; localized desquamation or other lesions covering <50% of body surface area (BSA)	Severe, generalized erythroderma or macular, papular or vesicular eruption; desquamation covering ≥50% BSA	Generalized exfoliative, ulcerative, or bullous dermatitis	Death
Urticaria Definition: A disorder characterized by an itchy skin eruption characterized by wheals with pale interiors and well-defined red margins.	• During or immediately after infusion • May be early indication of allergic reaction or anaphylaxis	Urticarial lesions covering <10% BSA; topical intervention indicated	Urticarial lesions covering 10-30% BSA; oral intervention indicated	Urticarial lesions covering >30% BSA; IV intervention indicated	-	-
Vascular disorders						
Hypertension Definition: A disorder characterized by a pathological increase in blood pressure; a repeatedly elevation in the blood pressure exceeding 140 over 90 mm Hg.	• During or immediately after infusion • Within 36 hours of infusion	Prehypertension (systolic BP 120 - 139 mm Hg or diastolic BP 80 - 89 mm Hg)	Stage 1 hypertension BP >ULN medical intervention indicated; recurrent or persistent (≥24hrs)	Stage 2 hypertension (systolic BP >=160 mm Hg or diastolic BP ≥100 mm Hg); medical intervention indicated; more than one drug or more intensive therapy	Life-threatening consequences (e.g., malignant hypertension, transient or permanent neurologic deficit, hypertensive crisis);	Death

				than previously used indicated	urgent intervention indicated	
Hypotension Definition: A disorder characterized by a blood pressure that is below the normal expected for an individual in a given environment.	• During or immediately after infusion • Within 36 hours of infusion	Asymptomatic, intervention not indicated	Non-urgent medical intervention indicated	Medical intervention or hospitalization indicated	Life-threatening and urgent intervention indicated	Death

Graft versus Host Disease [63]

Stage	**Skin**	**Liver (bilirubin)**	**Gut (stool output per day)**
0	No GVHD rash	< 2mg/dl	Child: < 10 ml/kg/day or persistent nausea
1	Maculopapular rash 25% BSA	2–3 mg/dl	Child: 10–19.9 ml/kg/day or persistent nausea, vomiting or anorexia, with a positive upper GI biopsy
2	Maculopapular rash 25–50% BSA	3.1–6 mg/dl	Child: 20–30 ml/kg/day
3	Maculopapular rash > 50% BSA	6.1–15mg/dl	Child: > 30 ml/kg/day
4	Generalized erythroderma plus bullous formation	> 15mg/dl	Severe abdominal pain with or without ileus
Grade			
I	Stages 1–2	None	None
II	Stage 3 or	Stage 1 or	Stage 1
III	—	Stages 2–3 or	Stages 2–4
IV	Stage 4 or	Stage 4	—