FORM-STAT-04A-01	Statistical Analysis Plan
Version 1.0	

Safety study of sibling cord blood cell infusion to children with cerebral palsy (SCUBI-CP)

SCUBI-CP Safety Protocol Version 10, 6th March 2017

Document Version History

Version Date	Version	Author	Signature	Change Description	Reason/Comment
30-01-2020	1.0	Rachel Schembri		Initial release	Not applicable

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LIST OF ABBREVIATIONS

AE	Adverse Event
ANCOVA	ANalysis of COVAriance
ANOVA	ANalysis Of VAriance
BP	Blood Pressure
CBU	Cord blood unit
CFCS	Communication Function Classification System
CP	Cerebral palsy
CRF	Case Report Form
DSMC	Data Safety Monitoring Committee
GCP	Good Clinical Practice
GMFCS	Gross Motor Function Classification System
GMFM	Gross Motor Function Measure
HLA	Human leucocyte antigen
HREC	Human Research Ethics Committee
ITT	Intent-To-Treat
LOCF	Last Observation Carry Forward
LS	Least Squares
MACS	Manual Ability Classification System
MedDRA	Medical Dictionary for Regulatory Activities
QUEST	Quality of Upper Extremity Skills Test
RCH	Royal Children's Hospital
SAE	Serious Adverse Event
SD	Standard Deviation
SE	Standard Error
TGA	Therapeutic Goods Administration
UCBC	Umbilical cord blood cell
WHO DD	World Health Organization Drug Dictionary

1. STUDY OBJECTIVES

1.1. PRIMARY OBJECTIVE

The primary objective of this study is to gain preliminary information on the safety of 12/12 Human leucocyte antigen (HLA) matched sibling umbilical cord blood cell (UCBC) infusion in children with cerebral palsy (CP).

1.2. 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

2. BACKGROUND/INTRODUCTION

2.1. STUDY DESIGN

This is a multi-site single group safety study of 12 participants with CP and matched sibling UCBCs. Sibling donor UCBCs require 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 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.

2.2. TREATMENT GROUPS

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.

2.3. STUDY POPULATION

Inclusion Criteria

- 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 if possible

Exclusion Criteria

- 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 107 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

2.4. INTERVENTION

HLA matched sibling UCBC infusion:

Intravenous (foot, hand or antecubital fossa)
Catheter size is a 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 x 10^7 cells/kg body mass (in line with other international trials)

2.5. SAMPLE SIZE

This trial will screen up to 48 potential 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 across 2 visits to determine formal enrolment into the study. 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. Screening of the 48 participants will be staggered to allow sequential assessment. Up to 12 of the 48 screened participants will formally be enrolled onto the trial after visit 2. The trial treatment and analysis will involve the 12 formally enrolled 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.

2.6. STUDY PROCEDURE

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. 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. Potential participants will then give a blood sample for tissue typing to match with UCBCs and (participant and sibling) saliva samples for genotyping.

Potential participants will be required to attend a second screening visit at the infusion hospital prior to infusion to confirm eligibility to be formally enrolled onto the study and establish baseline assessments. Following the confirmation of CP, enrolled participants will be invited for a third visit at the infusion hospital, where they will undergo the intervention (infusion). Participants will return to the hospital the next day following infusion 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. Follow up assessments will take place at 1 day, 1 week, 1 month, 3 months, 6 months, and 12 months post-infusion.

3. POPULATIONS OF ANALYSIS

Data will be presented for all 12 study participants.

4. OUTCOME VARIABLES

4.1. PRIMARY OUTCOME

The primary study objective is safety, and will be reported using on the following outcome measures:

Measure: Safety	Variable type	Description	Data presented as:
Vitals			
Count of out of range vitals	Binary	Count of AEs for out of range vitals (measured to 4 hours post infusion)	Summary per person (Median, IQR); Summary across all participants: N(%)
Pulse oximetry			
Count of out of range pulse oximetry	Binary	Count of AEs for out of range pulse oximetry (measured to 4 hours post infusion)	Summary per person (Median, IQR); Summary across all participants: N(%)
Markers of immune response			
Fraction of CD3+ T cells	Continuous	Fraction of cells (%) relative to their reference range (reported both as independent summary data and	Summaries across participants: 4hrs M(SD), Day 1 M(SD), Week 1

		relative to the reference range). Measured by: pbl_tcells (at 4hrs, Day 1, Week 1, 1 and 3 month timepoints)	M(SD), 3 month M(SD)
CD3+ / CD4+ T help cells	Continuous	Fraction of cells (%) relative to their reference range (reported both as independent summary data and relative to the reference range). Measured by: pbl_thelp (at 4hrs, Day 1, Week 1, 1 and 3 month timepoints)	Summary: 4hrs M(SD), Day 1 M(SD) , Week 1 M(SD), 3 month M(SD)
CD3+ / CD8+ cytotoxin T cells	Continuous	Fraction of cells (%) relative to their reference range (reported both as independent summary data and relative to the reference range). Measured by: pbl_tcytotox (at 4hrs Day 1, Week 1, 1 and 3 month timepoints)	Summary: 4hrs M(SD), Day 1 M(SD) , Week 1 M(SD), 3 month M(SD)
Natural Killer CD3- / CD16 & 56+	Continuous	Fraction of cells (%) relative to their reference range (reported both as independent summary data and relative to the reference range). Measured by: pbl_nk (at 4hrs, Day 1, Week 1, 1 and 3 month timepoints)	Summary: 4hrs M(SD), Day 1 M(SD), Week 1 M(SD), 3 month M(SD)
AEs			
Number of adverse events experienced by an individual	Count	Count of all adverse events experienced from infusion visit through 36 hours 3 & 12 months following infusion, by sub-category. Derived from the variables: ae_onset ae_category	Summary per person (Median, IQR); Summary across all participants: N(%)
Number of serious adverse events	Count	Count of serious adverse events experienced from infusion visit through 36 hours 3 & 12 months following infusion. ae_onset ae_serious	Summary per person (Median, IQR); Summary across all participants: N(%)

4.2. SECONDARY OUTCOMES

The secondary objectives are assessing the treatment effect, and time that the infused matched sibling UCBCs remain within the recipient, and will be reported based on the following measures:

Measure	Variable type	Description	Data presented as: (summary, individual, graphical)
Motor function			
Change from baseline in	Continuous	Total score (gmfm_gmae) and	Summary: M(SD) change

GMFM-66 at 3 and 12 months		percentile (gmfm_pctl)	& 95% CI
Change from baseline in QUEST at 3 and 12 months	Continuous	Domain B and total score	Summary: M(SD) change & 95% CI
Cognitive assessment			
Change in Bayley Scales of Infant Development, second edition, from baseline to 12 months. In 1-2 year old children only.	Continuous	z-score	List individually
Change in Wechsler Preschool Primary Scale of Intelligence, fourth edition from baseline to 12 months. In 2-6 year old children only.	Continuous	z-score	List individually
Change in Wechsler Intelligence Scale for Children from baseline to 12 months. In 6-16 year old children only.	Continuous	z-score	Summary: M(SD) change & 95% CI
Other			
Change in Beery-Buktenica Developmental Test of Visual-Motor Integration from baseline to 12 months	Continuous	Total score and percentile	Summary M(SD) change & 95% CI
Change in Vineland Adaptive Behaviour Scales from baseline to 12 months	Continuous	Composite score	Summary M(SD) change & 95% CI
Change in Behaviour Rating Inventory of Executive Function from baseline to 12 months	Continuous	Total score from either the BRIEF or BRIEF-P, ie [brief_gec] and [briefp_gec] (slightly different measures with the same scoring and same interpretation)	Summary M(SD) change & 95% CI
Change in Strengths and Difficulties Questionnaire from baseline to 12 months	Continuous	Total score, from either SDQ or mSDQ ie [sdq_total] and [sdqm] (slightly different measures with the same scoring and same interpretation)	Summary M(SD) change & 95% CI
Change in CP-QoL CHILD from baseline to 12 months	Continuous	Domain scores, ie [cpqol_a_v6], [cpqol_b_v6], [cpqol_c_v6], [cpqol_d_v6], [cpqol_e_v6], [cpqol_f_v6], [cpqol_g_v6].	Summary M(SD) change & 95% CI
Measure: Length of time infusion remains within	**Variable type**	**Description**	

recipients			
How long donor DNA was present in recipient	Categorical	Length of time calculated as time from infusion to most recent date where "Was donor DNA present?" was YES (in days?). Derived from: V3_date (infustion) and pbl_chim (and the date of this time point), then classified into categories. Categories: 'immediate clearance' to indicate return to baseline fraction of foreign cell-free DNA within 24 hours; 'clearance' to indicate return to baseline fraction of foreign cell-free DNA by 1 month; 'slow clearance' to indicate the presence of between 200 donor genome equivalent/ml and engraftment at 3 months, and 'engraftment'.	Summary: N(%)
How long donor DNA was present in recipient	Continuous	The most recent date where "Was donor DNA present?" was YES. Derived from: pbl_chim (and the date of this time point), presented as number of weeks	Summary: M(SD)

Safety data will be summarised as the proportion of participants who have 1) an SAE and 2) an AE within each of the three safety 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. Change in motor and cognitive function at 12 months will be presented relative to baseline, presented as a mean change in each outcome.

4.3. OTHER PARAMETERS

DEMOGRAPHY AND BASELINE

Demographic data will be presented as M(SD) or N(%) for:

Participant characteristics	Type	Variable name	Summary
Sex	Binary	sex	N(%)
Age at infusion	Continuous	age_infuse (or derive from v3_date – dob)	M(SD)
Gross Motor Function Classification	Binary	Derived from gmfcs (I/II/III vs IV/V)	N(%)
Manual Ability Classification	Categorical (5)	Derived from macs (I/II/III vs IV/V)	N(%)
Communication Function Classification	Categorical (5)	Derived from cfcs (I/II/III vs IV/V)	N(%)
Primary motor type	Categorical (5)	type	N(%)

Limbs affected	Categorical (4)	cp_descript	N(%)
Brain pathology	Categorical (4)	mri_category	N(%)
Seizure disorder	Binary	seizure	N(%)
Possible intellectual impairment	Categorical	Combination of perceptual and (iq and bsid_copg_pcr) and cog_tool_none	N(%)
Blood group	Categorical	abo and rhesus	This should only be presented if describing participants individually
Umbilical Cord blood unit (UCBU) characteristics			
Blood group of CBU		cbu_abo and cbu_rhesus	This should only be presented if describing participants individually
ABO mismatch	Binary	Calculated by comparing cbu_abo and abo	N(%)
TNC count in washed CBU ($x10^7$)	Continuous	tnc	Median (min,max)
Haemopoietic stem cell (CD34+) count in washed CBU ($x10^6$)	Continuous	cd34count	Median (min,max)
Predicted# TNC/kg ($x10^7$)	Continuous	dose_tnc_pilot	Median (min,max)
Predicted# CD34+ cells/kg ($x10^6$)	Continuous	dose_cd34_pilot	Median (min,max)
Actual TNC/kg ($x10^7$)	Continuous	dose_tnc	Median (min,max)
Actual CD34+ cells/kg ($x10^6$)	Continuous	dose_cd34	Median (min,max)

5. STATISTICAL METHODOLOGY

5.1. GENERAL METHODOLOGY

Given the pilot nature of this trial, the results from this study will be presented descriptively. The distribution of all continuous measures will be observed and will be reported as Median and interquartile range rather than M(SD) if necessary. Data will be presented as a list (individual data) rather than summary data if n=3 or less due to missing data, and for all cognitive measures.

HANDLING OF MISSING DATA

No statistical techniques will be used to account for missing data due to the pilot / descriptive nature of the results to be presented. A summary of how much data was missing, and how much of that was due to lack of testing due to feasibility challenges will be presented by participant.

SENSITIVITY ANALYSES

No sensitivity analyses will be conducted.

SUBGROUP ANALYSIS

Some graphs of GMFM and QUEST will highlight more and less severe cases in different colours, as outlined below in Section 5.3.

CLASSIFICATION OF PROTOCOL VIOLATION

No participants will be removed from the analysis due to protocol violations. Protocol violations will be reported descriptively, as appropriate.

5.2. ANALYSIS OF PRIMARY OBJECTIVE

The primary objective is around safety over the first 36 hours following infusion, as outlined in the table of primary outcomes. Outcomes will be reported for the 12 individual cases if required for further detail.

5.3. ANALYSIS OF SECONDARY OBJECTIVES

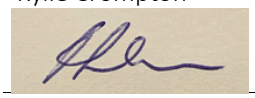
Safety outcomes will be presented at 3-month and 12-month time points (secondary outcomes) using the same methodology as the 36 hour data (primary outcome), as outlined in the table of secondary outcomes. Outcomes will be reported for the 12 individual cases if required for further detail.

Graphs will also be produced to display the relationships of interest amongst variables:

Type	Y-axis (outcome)	X-axis	Time point	Subgroup (if applicable)
Scatterplot with line of best fit	Cell persistence (continuous)	BMI (continuous)		N/A
	Cell persistence (continuous)	baseline lymphocyte count (continuous)		N/A
	Cell persistence (continuous)	blood group mismatch (binary)		N/A
	Cell persistence (continuous)	TNC dose (continuous)		N/A
	Cell persistence (continuous)	CD34 dose (continuous)		N/A
Line graph	Mean GMFM66 score	Timepoints (baseline, 3, 12 months)	All	N/A
Line graph	Individual GMFM66 centiles (continuous)	Timepoint (baseline, 3, 12 months)	All	Showing children treated before or after they reached their 90% motor threshold in different colours
Line graph	Individual GMFM66 centiles (continuous)	Timepoint (baseline, 3, 12 months)	All	Showing children with concomitant rehab therapy above or below a threshold (variable ther_category) in different colours
Scatterplot with line of best fit	Change in GMFM66 centile at 3 months	TNC dose (continuous)	3mo	N/A
Scatterplot with line of best fit	Change in GMFM66 centile at 12 months	TNC dose (continuous)	12mo	N/A
Scatterplot with	Change in GMFM66	Cell persistence	3mo	N/A

line of best fit	centile at 3 months	(continuous)		
Scatterplot with line of best fit	Change in GMFM66 centile at 12 months	Cell persistence (continuous)	12mo	N/A
Line graph	Individual QUEST score	Timepoint (baseline, 3, 12 months)	All	Showing children able or unable to grasp (variable quest_grasp) in different colours
Line graph	Individual QUEST score	Timepoint (baseline, 3, 12 months)	All	Showing children with concomitant rehab therapy above or below a threshold (variable ther_category) in different colours
Scatterplot with line of best fit	Change in QUEST score at 3 months	TNC dose (continuous)	3mo	N/A
Scatterplot with line of best fit	Change in QUEST score at 12 months	TNC dose (continuous)	12mo	N/A
Scatterplot with line of best fit	Change in QUEST score at 3 months	Cell persistence (continuous)	3mo	N/A
Scatterplot with line of best fit	Change in QUEST score at 12 months	Cell persistence (continuous)	12mo	N/A
Bar graph	Mean change in QoL pain score (cpqol_f_v6) (continuous)	Timepoint (baseline, 3, 12 months)	All	Dichotomised severity (2 categories, GMFCS I+II+III versus IV+V)
Bar graph	Mean change in QoL access to services score (cpqol_e_v6) (continuous)	Timepoint (baseline, 3, 12 months)	All	Dichotomised severity (2 categories, GMFCS I+II+III versus IV+V)
Scatterplot with connected line	Fraction of CD3+ T cells (overlayed with Haematocrit [pbl_hct] and Sodium [pbl_Na] as references).	Timepoint (baseline, 4hrs, Day 1, Week 1, 1 and 3 months)	All	Three lines: CD3+ T cells, Haematocrit and Sodium
	CD3+ / CD4+ T help cells (overlayed with Haematocrit [pbl_hct] and Sodium [pbl_Na] as references).	Timepoint (baseline, 4hrs, Day 1, Week 1, 1 and 3 months)	All	Three lines: CD3+ / CD4+ T help cells, Haematocrit and Sodium
	CD3+ / CD8+ cytotoxic T cells (overlayed with Haematocrit [pbl_hct] and Sodium [pbl_Na] as references).	Timepoint (baseline, 4hrs, Day 1, Week 1, 1 and 3 months)	All	Three lines: CD3+ / CD8+ cytotoxic T cells, Haematocrit and Sodium

6. SIGNATURES

Signature of Investigator, on behalf of Principal Investigator:	Kylie Crompton.	20 July 2020 Date
Print Name	Kylie Crompton	
Signature of Trial Statistician:		Date 20/7/2020
Print Name	Dr Rachel Schembri	