

Statistical Analysis Plan

**A Randomised Controlled Trial of Red Cell Washing for the Attenuation of
Transfusion Associated Organ Injury in Cardiac Surgery**

(REDWASH)

SAP Version: 0.8
Date: 13-Jul-2015

Based on protocol / protocol amendment : REDWASH-00
Version: 3.0
Date: 04-Aug-2013

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Trial registration

MREC No 12/EM/0475
ISRCTN 27076315
UKCRN ID 13922

Revision History (only circulated versions)

Version	Date	Author(s) and Role	Summary of Changes/Comments
0.1	12-MAY-2015	Tom Morris (Trial Statistician)	Initial version
0.3	29-MAY-2015	Tom Morris Gavin Murphy	Inclusion of GM comments following SAP meeting on 20/05/2015.
0.6	10-JUN-2015	Tom Morris Gavin Murphy	Further comments, and corrections to time points.
0.8	13-JUL-2015	Tom Morris Gavin Murphy Cassey Brookes	Inclusion of CB comments.

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LIST OF ABBREVIATIONS

AE	Adverse Event
AKI	Acute Kidney Injury
ARDS	Acute respiratory distress syndrome
BRU	Biomedical Research Unit
CI	Chief Investigator
CPB	Cardiopulmonary Bypass
CRF	Case Report Form
CTP	Clinical Trial Protocol
CTR	Clinical Trial Report
CTU	Clinical Trials Unit
FDA	Food and Drug Administration
GM-CSF	Granulocyte macrophage colony stimulating factor
HDU	High Dependency Unit
ICH	International conference of Harmonisation
ICU	Intensive Care Unit
IFN- γ	Interferon gamma
IL	Interleukin
ITT	Intention-to-treat
ITU	Intensive Therapy Unit
KDIGO	International Kidney Diseases: Improving Global Outcomes
LFABP	Renal liver-type fatty acid binding protein
MI	Myocardial infarction
MODS	Multiple Organ Dysfunction Score
NGAL	Neutrophil gelatinase-associated lipocalin
NHSBT	NHS Blood and Transplant
PPS	Per protocol set
RBC	Red Blood Cells
SAE	Serious Adverse Event
SAF	Safety population
SAP	Statistical Analysis Plan
SIRS	Systemic Inflammatory Response Syndrome
SUSAR	Suspected Unexpected Serious Adverse Reaction
TNF- α	Tumour necrosis factor alpha

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1 Introduction

This Statistical Analysis Plan (SAP) describes the planned analysis and reporting for the trial Murphy_12_130 REDWASH (protocol REDWASH-001). The structure and content of this SAP provides sufficient detail to meet the requirements identified by the FDA and International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH): Guidance on Statistical Principles in Clinical Trials. All work planned and reported for this SAP will follow internationally accepted guidelines, published by the American Statistical Association and the Royal Statistical Society for statistical practice.

The reader of this SAP is encouraged also to read the clinical protocol for details on the conduct of this study, and the operational aspects of clinical assessments and timing for the process of completing a patient in this study.

The purpose of this SAP is to outline the planned analyses that are to be performed on the data to support the completion of the Clinical Trial Report (CTR). The SAP will be amended if there are substantial changes to the planned analyses, and in any case, will be finalized before the database lock for this study. Exploratory post-hoc or unplanned analyses not necessarily identified in this SAP may be performed on these data as required. These analyses will be clearly identified in the CTR.

Throughout the document: Any verbatim text from the protocol is provided in italics inside a border:

<i>Text from the protocol.</i>

1.1 Study Objectives

The REDWASH Trial proposes to test the hypothesis that that the severity of the postoperative inflammatory response will be less and post-operative recovery faster if patients undergoing cardiac surgery with CPB who are at risk of massive RBC transfusion receive stored allogenic RBC that are washed prior to transfusion when compared to standard care where stored RBC are administered without washing.

1.1.1 Primary Objectives

A. Estimate mean differences in biochemical markers of the systemic inflammatory response between participants allocated to receive washed versus unwashed RBC.

1.1.2 Secondary Objectives

A secondary hypothesis is that the adverse effects of transfusion are mediated by platelet and monocyte activation by microparticles within the storage supernatant and that by removing the supernatant this is attenuated.

...

B. Estimate mean differences in hospital length of stay between participants allocated to receive washed versus unwashed RBC.

C. Estimate differences in the frequency of inflammatory organ injury or death between participants allocated to receive washed versus unwashed RBC.

D. Estimate the cost-effectiveness of washed versus unwashed RBC.

E. Establish whether red cell washing attenuates postoperative platelet and monocyte activation (subgroup analysis).

1.1.3 Exploratory Objectives

None.

1.2 Study Design

This study is a multi-centre, randomised, single blinded parallel group, randomised controlled trial of washing of allogenic RBC prior to transfusion versus standard care (no washing).

1.2.1 Study Interventions

Eligible patients undergoing cardiac surgery with CPB who consent to participate will be randomly allocated, in a 1:1 ratio to:

- 1. GROUP A: Unwashed RBC (standard care)*
- 2. GROUP B: Washed RBC*

1.2.2 Intervention Group

Allogenic RBC, harvested in citrate-adenine-phosphate-dextrose, buffy coat removed, leucocyte depleted, saline-adenine-glucose-mannitol stored red cell units, supplied by NHSBT as per standard practice will be used. For the intervention each unit of RBC will be added to a Continuous AutoTransfusion System, washed using a centrifugal method, as per the device instructions. The washed RBC will then be immediately administered to the patient as per standard practice.

1.2.3 Control Group

Allogenic RBC, harvested in citrate-adenine-phosphate-dextrose, buffy coat removed, leucocyte depleted, saline-adenine-glucose-mannitol stored red cell units, supplied by NHSBT as per standard practice will be used.

1.2.4 Sample size

The primary outcome, serum IL8 levels, is continuously scaled, so the target differences can be specified as a “standardised differences” (0.2=small, 0.5=moderate, 0.8=large). On the assumption that there will be a moderate correlation of 0.7 between pre and post-intervention measures and between repeated post-intervention measures, as observed in previous work [35], and on the basis that there will be one baseline and three postoperative measures, we estimate that a sample size of 150 patients will allow us to detect a small to moderate target difference between groups of 0.4, with 90% power and 5% significance (2-tailed). We propose to recruit 170 patients (85 per group) assuming an attrition rate of between 10-15% for incomplete sampling, patient death and withdrawal. The sample size will also allow us to detect a 40% difference in the composite endpoint of any sepsis, inflammatory organ injury or

death with 80% power and 5% significance. For time to ICU / hospital discharge, the sample size will allow a hazard ratio of 1.65 to be detected.

1.2.5 Blinding

...the investigators, including those responsible for the collection of postoperative data and laboratory and statistical analyses will be blinded.

The analysis will initially be conducted using masked treatment groups and with data that could lead to unblinding removed (blood data, adverse events). Once this initial analysis is complete, the treatment groups will be revealed and the remaining data added to the analysis.

1.3 Schedule of Major Assessments

	Pre-Op'n	Op'n Day	Day 1	Day 2	Day 3	Day 4	Dis-charge	6 wks	3 months
Eligibility	✓								
Written consent	✓								
Randomisation	✓								
EQ5D Questionnaire	✓						✓	✓	✓ <u>U</u>
Bloods: serum biochemistry (creatinine and troponin T/I)	✓ †	✓ † (CICU & 6 -12 hrs)	✓ † (24 hrs)	✓ † (48 hrs)	✓ † (72 hrs)	✓ λ (96 hrs)	✓ λ		
Bloods: serum inflammatory biomarkers	✓ †	✓ † (CICU & 6 -12 hrs)	✓ † (24 hrs)	✓ † (48 hrs)					
Bloods: Full blood counts	✓	✓ (CICU & 6 -12 hrs)	✓ (24 hrs)	✓ (48 hrs)	✓ (72 hrs)	✓ λ (96 hrs)	✓ λ		
Bloods: Plasma sample for MP analysis and monocyte activation	✓	✓ (CICU)	✓ (24 hrs)	✓ (48 hrs)					
Urine sample & volume: NGAL, urea and electrolytes	✓	✓ (6 & 12 hrs)	✓ (24 hrs)	✓ † α (48 hrs)					
Operative details		✓ †							
Clinical outcomes						✓	✓	✓ \S	
Serious adverse event monitoring		✓	✓	✓	✓	✓	✓	✓ \S	
Resource use data							✓	✓ \S	✓ <u>U</u>
Bloods: Platelet response \yen	✓ \yen	✓ \yen (CICU & 6 -12 hrs)	✓ \yen (24 hrs)	✓ \yen (48 hrs)					

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^ Discharge time point if hospital stay exceeds 5 days

§ 4-6 week time point in accordance with normal postoperative care.

† Indicates samples taken as part of normal care.

α Indicates sample for determination of routine urea and electrolytes only

¥ Indicates Glenfield patients alone

∩ Indicates data collection via postal questionnaire

1.4 Changes to the Planned Analysis

The study has been stopped prematurely by the funder because of slow recruitment. The final analysis will now take place, with 60 consented patients.

The primary outcome is confirmed as serum cytokine IL-8 with repeated measures at four post-surgery time points: on return to ITU, and 6-12 hours, 24 and 48 hours post-operatively.

ARDS is now defined according to the Berlin definition [Ranieri, 2012] as PaO₂/FiO₂ ratio < 300 mmHg where the Continuous Positive Airway Pressure (CPAP) or Positive End Expiratory Pressure (PEEP) is > 5cmH₂O.

Blood loss is recorded at 4 hours and 12 hours, rather than the 6 hours given in the protocol.

The study did not measure GM-CSF, IFN- γ , IL-1 β , IL-2, IL-4, IL-5, and IL-10, as stated in the protocol. Levels of IL-6 and TNF- α were recorded as planned and MIP-1 and MCP-1 were recorded in addition.

2 Outcomes

A complete list of variables to be evaluated in the statistical analysis is provided in a separate document, "REDWASH outcomes v0.3".

2.1 Primary Outcomes

2.1.1 Definition of Primary Outcomes

The primary outcome for the trial is the severity of the systemic inflammatory response as indicated by serum cytokine levels IL8. These will be measured from venous blood samples taken preoperatively, on return to ITU, and 6 hours, 24, 48 and 96 hours post-operatively.

2.1.2 Hypotheses to be investigated

The primary hypothesis is that serum cytokine IL-8 will be reduced by transfusion with washed red blood cells in comparison to unwashed red blood cells over the first 96 hours post-operatively.

The difference in serum cytokine IL-8 will also be investigated at four time points individually, namely return to ITU, 6-12, 24 and 48 hours post-operatively.

2.1.3 Handling of Missing Data

Any missing outcome and cost data will be dealt with using multiple imputation methods.

Lab data below the limit of quantification will be imputed as half the limit of quantification.

2.2 Secondary Outcomes

2.2.1 Definition and Derivation of Secondary Outcomes

In addition we will measure serum levels of GM-CSF, IFN- γ , IL-1 β , IL-2, IL-4, IL-5, IL-6, MCP-1, MIP-1 IL-10 and TNF- α .

Serum levels of MIP-1 and MCP-1 were measured in place of GM-CSF, IFN- γ , IL-1 β , IL-2, IL-4, IL-5 and IL-10. Serum levels of IL-6 and TNF- α were recorded as planned

(a) Inflammatory Organ Injury, Sepsis or Death:

- Sepsis will be defined as Antibiotic treatment for suspected infection, **and** the presence of SIRS within 24 hours prior to start of antibiotic treatment*

*where SIRS is defined as ≥ 2 of the following conditions: temperature $> 38^{\circ}\text{C}$ or $< 36^{\circ}\text{C}$; heart rate > 90 beats / min; respiratory rate > 20 breaths / min or $\text{PaCO}_2 < 32$ mmHg; white blood cell count $> 12,000 / \text{mm}^3$ or $< 4,000 / \text{mm}^3$, **or** antibiotic treatment for wound infection.*

- *Acute Kidney Injury, defined as KDIGO stage 1, 2 or 3.*
- *Acute lung injury, defined as $\text{PaO}_2/\text{FiO}_2$ ratio < 300 mmHg or a requirement for respiratory support; invasive ventilation > 48 hours, non-invasive ventilation > 4 hours, reintubation, tracheostomy, or ARDS.*
- *Low cardiac output, defined as new intra-or postoperative intra-aortic balloon pump insertion or a cardiac index of $< 2.2 \text{ L} \cdot \text{min}^{-1} \cdot \text{m}^{-2}$ refractory to appropriate intravascular volume expansion after correction or attempted correction of any dysrhythmias, or the administration of inotropes including Dobutamine, Enoximone, Milrinone, Levosimendan and Adrenaline.*
- *Death*
- *Differences in Multiple Organ Dysfunction Score [36] at days 1, 2, 3 and 5.*

Sepsis is defined as at least one of the following:

- antibiotic treatment for suspected infection AND presence of SIRS
- antibiotic treatment for wound infection.

Since the publication of the protocol, a new consensus definition (the Berlin definition) of ARDS has been published [Ranieri, 2012]. This replaces both the old definition of ARDS and the term “acute lung injury”, which has been abandoned. ARDS is now defined as $\text{PaO}_2/\text{FiO}_2$ ratio < 300 mmHg where the Continuous Positive Airway Pressure (CPAP) or Positive End Expiratory Pressure (PEEP) is > 5 cmH₂O. ARDS will be defined using the Berlin criteria as Mild, Moderate or Severe.

Due to this change, the outcome “requirement for respiratory support” will not be reported.

The inotropes included in the definition of low cardiac output are an exhaustive list.

Each of the six components of the Multiple Organ Dysfunction Score (MODS) will be an outcome, as well as forming the overall MODS outcome. See section 4.1 for the derivation of the MODS.

The sample size will allow us to detect a 40% difference in the composite endpoint of any sepsis, inflammatory organ injury or death...

A composite outcome consisting of sepsis, acute kidney injury, ARDS, low cardiac output and death will be derived.

(b) *Bleeding and Transfusion*

- *Blood loss at 6 hours postoperatively.*
- *The number of units of RBC and other blood components transfused during the operative period and post-operative hospital stay will be recorded*

Blood loss is recorded at 4 hours and 12 hours, rather than the 6 hours given in the protocol.

(c) *Transfusion Reactions*

- *Febrile Transfusion Reactions*
- *Non-haemolytic transfusion reactions.*
- *Haemolytic Transfusion reactions.*

The transfusion reactions are potential adverse events and will be recorded only on the adverse event log. Since these will be entered as free-text, the Chief Investigator will confirm which adverse events can be categorised as one of these reactions on a case-by-case basis.

(d) *Other clinical outcomes*

- *Stroke; diagnosed by brain imaging (CT or MRI), in association with new onset focal or generalised neurological deficit (defined as deficit in motor, sensory or co-ordination functions)*
- *ST elevation myocardial infarction accompanied by troponin I > 5000 pg / ml*

(e) *Hospital stay and cumulative resource use*

- *ITU, HDU and hospital length of stay will be determined by the assessment of care level (see section 5.12 and reference 37).*
- *Resource use will be costed using credible nationally published sources.*
- *An incremental cost per complication avoided will be calculated (further details in section 6.4).*

The following time-to-event outcomes will be evaluated:

- ventilation time / time to extubation
- time to discharge from CICU/HDU
- time to discharge from hospital

Ventilation time is defined as the time between return from theatre and extubation.

Time to discharge from CICU/HDU is defined as the sum of the time between admission to CICU/HDU and admission to ward, and the time between return to CICU/HDU and return to ward. Note that if there was no return to CICU/HDU then this second time period is 0.

Time to discharge from hospital is defined as the time from return from theatre to discharge.

All time are measured in hours.

No resource use or cost analyses will be conducted due to the early termination of the study. The EQ-5D was recorded to be part of the intended health economic analyses, and this will now be presented instead as part of the statistical analysis.

(f) Compliance with the washing protocol

- *Data will be collected for all patients during surgery to characterise compliance with the randomly assigned washing protocol.*

(g) Additional markers of inflammation and organ injury

- *Urinary Liver Fatty Acid Binding Protein (LFABP), Neutrophil gelatinase associated lipocalin (NGAL) at baseline and at 6, 12 and 24 hours for a sub-group of patients (n= 40 per group).*
- *Serum Troponin I at baseline and at 24 and 48 hours.*
- *Platelet aggregation (Multiplate®) in the first 48hrs for a sub-group of patients (patients at Glenfield hospital only).*
- *Age of each unit of RBC transfused.*
- *Transfused RBC characteristics (washed and unwashed); Adenosine Triphosphate (ATP) levels, 2,3DPG, deformability, osmotic fragility, cytokine levels.*
- *Platelet and monocyte activation as determined by flow cytometry for a sub-group of patients (patients at Glenfield hospital only).*
- *Endothelial injury as determined by quantification of endothelial derived microparticles (as per figure 3) by flow cytometry.*
- *Effect of blood harvested from recipients on platelet and monocyte activation within a microfluidics system, as per figure 5)*

Due to the lack of funding, the following outcomes were not recorded:

- LFABP
- transfused RBC characteristics (except age of each unit)
- effect of blood on platelet and monocyte activation

NGAL was measured in all patients, not a subset.

Serum troponin I was measured at baseline and on return to ITU, 6-12, 24, 48, 72 and 96 hours post-operatively.

Platelet aggregation was measured at baseline and on return to ITU, 6-12, 24 and 48 hours. This outcome, together with endothelial injury, will be analysed in an exploratory fashion separate to the clinical trial analyses. The results from these external analyses will be presented together with the clinical trial results in a joint publication. See section 8.1.

Creatinine clearance will be an additional outcome, calculated as in section 4.1.

2.2.2 Hypotheses to be investigated

All secondary outcomes are hypothesised to benefit from the intervention.

2.2.3 Handling of Missing Data

Any missing outcome and cost data will be dealt with using multiple imputation methods.

Lab data below the limit of quantification will be imputed as half the limit of quantification.

When individual components of the MODS are missing, imputation rules are built in to the definition of the score.

2.3 Other Efficacy Outcomes

None.

2.4 Other Outcomes of Interest

Compliance with the washing protocol.

2.4.1 Handling of Missing Data

If historic dates are not fully known (e.g. medical history), the following rules for data entry will be applied at data entry:

- If Month and Year are known, but not the exact date, the 15th will be used as imputation rule.
- If only the Year is known, the 01-Jul will be used as imputation rule.

3 Analysis Sets/Populations

3.1 Protocol deviations (PD)

Time window violations are generally not considered as major protocol deviations.

Patients who underwent randomisation but did not undergo surgery will not be considered in the analysis. Patients entered into the trial in error (found post randomisation and surgery to be ineligible) will be included in the safety population only.

Receipt of only unwashed blood for patients randomised to receive washed blood will be considered a major protocol violation.

Receipt of some units of unwashed blood in a patient randomised to receive washed blood, and who receive at least 1 unit of washed blood, will be considered a minor protocol violation. Patients not followed up to the end of the trial (3 months) will be considered minor protocol violations. Patients with missing data that relate to laboratory and biochemical values will also be considered as minor protocol violations.

3.2 Intention-to-treat Population / Full analysis set (ITT/FAS)

<i>The trial will be analysed on an intention-to-treat basis.</i>

The intention-to-treat population comprises all patients randomised into the trial who underwent surgery, with patients considered to be in the group they were randomised to, regardless of the treatment they eventually received.

3.3 Per-protocol sets (PPS)

The per-protocol population comprises all patients who have been recruited into the trial, had the trial intervention administered and who do not have major protocol deviations.

3.4 Safety Population (SAF)

The safety population comprises all patients who have been recruited into the trial and had the trial intervention administered. In this population, patients are considered to be in the intervention group if they received at least one unit of washed blood. Otherwise, they are considered to be in the control group. The SAF will be used for all safety analyses.

3.5 *Other Analysis Populations*

Not applicable.

4 General Issues for Statistical Analysis

4.1 *Derived/ Computed Variables*

Sepsis

Sepsis is defined as at least one of the following:

- antibiotic treatment for suspected infection AND presence of SIRS
- antibiotic treatment for wound infection

where SIRS is defined as at least two of the following:

- temperature > 38°C or < 36°C
- heart rate > 90bpm
- respiratory rate > 20 breaths per minute OR PaCO₂ < 32mmHg
- white blood cell count > 12,000/mm³ or < 4,000/mm³

ARDS

ARDS is defined according to the new Berlin definition [Ranieri, 2012].

Creatinine clearance

Creatinine clearance will be calculated by the formula

$$CCr [ml/min] = \frac{UCr [mmol/l] \times Vdt [ml/min]}{PCr [\mu mol/l] \times 1000}$$

where Creatinine Clearance (*CCr*) is calculated from the creatinine concentration in the collected urine sample (*UCr*), urine flow rate (*Vdt*), and the plasma concentration (*PCr*).

MODS

The MODS comprises six components which are objectively scored from 0 to 4, then summed to form a total from 0 to 24, where higher scores indicate higher organ dysfunction. In this study it was recorded at baseline and at six time-points during follow-up, and will be analysed using two methods. The first will be a repeated measures analysis consisting of the MODS at each time point. The second will be a derived “worst value” MODS that was recently used in the RECESS trial [Steiner, 2015], defined as follows^[gjm191]:

At time point $i = 0, \dots, 6$ (where 0 is baseline) and MODS component $j = 1, \dots, 6$ let M_{ij} be the score of the component, from 0 to 4. Then the worst-value score for MODS component j is

$$M^*_j = \max\{M_{ij} \mid i = 1, \dots, 6\}.$$

The overall worst-value MODS is then

$$MODS = \sum_{j=1}^6 M^*_j$$

and the change from baseline is

$$\Delta MODS = \sum_{j=1}^6 (M^*_j - M_{0j}).$$

Other continuous variables

In a similar manner, secondary analyses will be conducted on the continuous outcomes using “highest values” and “lowest values” over time. Specifically, for a continuous outcome y_i measured at time points $i = 1, \dots, n$, the following outcomes will be derived:

$$\max\{y_i \mid i = 1, \dots, n\} \text{ and } \min\{y_i \mid i = 1, \dots, n\}.$$

EQ-5D

For the EQ-5D-3L questionnaire, a summary score will be calculated at each time point for each patient. This will be based on the VAS index derivation [Euroqol].

	VAS value set UK
Full health (11111)	1
At least one 2 or 3 (constant)	-0.155
At least one 3 (N3)	-0.215
Mobility = 2	-0.071
Mobility = 3	-0.182
Self care = 2	-0.093
Self care = 3	-0.145
Usual activities = 2	-0.031
Usual activities = 3	-0.081
Pain/discomfort = 2	-0.084
Pain/discomfort = 3	-0.171
Anxiety/depression = 2	-0.063

Anxiety/depression = 3 -0.124

4.2 Multiplicity, Multiple Comparisons and Interim Analyses

The primary analysis will take place when follow-up is complete for all recruited patients. No formal interim analysis is planned. Outcome data will be reported to the Data Monitoring and Safety Committee every 6 months, together with any additional analyses the committee request. In these reports the data will be presented by group but the allocation will remain masked.

The study has been stopped prematurely and the final analysis will now take place, with 60 patients having been recruited, of whom 56 have undergone surgery.

There will be no adjustment for multiple comparisons.

4.3 Planned Subgroups, Interactions and Covariates

Two sensitivity analyses are planned, comparing the primary outcome for ... 2. Patients who receive older blood versus younger blood, i.e. those who receive only blood less than 14 days old versus those that receive any blood over 14 days old, on the basis that the proposed intervention is expected to prevent the risks attributed to prolonged blood storage.

These subgroup analyses will not be undertaken due to the small number of patients for whom data were recorded.

4.4 Analysis Software

The clinical data will be extracted from a MACRO data base. Laboratory data will be transferred from the lab sites to the CTU via validated EXCEL sheets.

The analysis will be performed with a current version of SAS™. Graphical displays will generally be generated using R, possibly using the graphics package “ggplot2”.

CRF Data without values might be coded as “not available” (by investigator) or “missing” (by data base). Any data which are derived from missing or not available data will however always be coded as “missing” only.

5 Statistical Methodology

The statistical analysis will be based on external guidelines (e.g. ICH E3 and E9), and displayed in accordance with the CTU display catalogue and other external recommendations [Bamnote, 2012].

The table of contents of all analyses will be planned on an EXCEL table, including the risk assessment and the validation methods of each display.

5.1 *The date of data extraction from the database will be included in all tables and listings. Disposition of Patients*

Patient disposition will be presented with respect to completion status, reason for non-completion and length of stay in trial. Results will be tabulated and summarised over time by treatment group and in total.

A CONSORT chart will display the flow of patients through the trial.

A graph of cumulative recruitment will be presented.

5.2 *Demographic and Baseline Characteristics*

The demographic and clinical characteristics and the medical history and concomitant medication will be tabulated and summarised by treatment group and in total. This will include the stratification variables.

Numbers (with percentages) for binary and categorical variables and means (and standard deviations), and medians (with lower and upper quartiles) for continuous variables will be presented.

There will be no formal comparison of baseline variables between treatment groups.

5.3 *Primary Outcome Analysis*

5.3.1 *Primary Analysis of Primary Outcomes*

The primary analysis will take place when follow-up is complete for all patients and will be performed on an intention-to-treat basis. Means for continuous outcomes (transformed logarithmically if required) will be compared using analysis of variance or regression modelling, adjusting for baseline values where available. Findings will be reported as effect sizes with 95% confidence intervals.

The primary analysis will be a repeated measures model. Each patient will contribute four (follow-up) repeated measures to the model, and a compound-symmetry covariance structure will be used to model within-patient correlation. The suitability of this structure was observed in previous work (reference 35 in

the protocol). The model will be adjusted for the outcome at baseline and the type of procedure. The other stratification variable, study site, will not be adjusted for since most of the randomised patients were recruited from a single site.

5.3.2 Secondary Analyses of Primary Outcomes

The primary outcome will be analysed at each of the four follow-up time-points separately. These analyses will be linear regression models, adjusted for the outcome at baseline and the type of procedure.

Two additional models will be fitted with the “highest value” and “lowest value” outcomes as defined in section 4.1.

5.3.3 Sensitivity and Exploratory Analyses

All analyses will be repeated with the per-protocol set.

5.3.4 Subgroup Analyses

Two sensitivity analyses are planned, comparing the primary outcome for ... 2. Patients who receive older blood versus younger blood, i.e. those who receive only blood less than 14 days old versus those that receive any blood over 14 days old, on the basis that the proposed intervention is expected to prevent the risks attributed to prolonged blood storage.

These planned subgroup analyses will not be carried out due to the small numbers of patients who completed the trial.

5.4 Secondary Outcome Analyses

The biomarkers IL-6, MIP-1, MCP-1 and TNF- α will be analysed by the same models as the primary outcome.

The MODS is a long-ordinal variable which will be treated as a continuous variable and analysed in a repeated measures model in the same manner as the primary outcome. In addition, MODS will be analysed according to the “worst-value” method used in [Steiner, 2015]. See section 4.1 for the definition of this derived score.

The EQ-5D summary score will also be treated as a continuous variable and analysed in a similar manner to the primary outcome.

All categorical variables will be analysed using logistic regression adjusted for the type of procedure.

Time to classification as fit for discharge, ICU and post-operative hospital stay will be analysed as time-to-event data using regression modelling for survival data.

5.5 Additional Efficacy Analyses

None.

6 Safety and Tolerability Analysis

6.1 Drug Exposure

Concomitant medication will be listed and summarised.

6.2 Adverse Events and Tolerability

Adverse events will be recorded and reported in accordance with the University of Leicester's and University Hospitals Leicester NHS Trust's policies for reporting Research Related Adverse Event.

In cardiac surgery, post-operative transient complications are not unexpected and are not infrequent. The research team will only notify deaths and 'unexpected' non-fatal SAEs to the Trial Sponsor (University of Leicester Research Support Office). Unexpected events are those not listed in the trial protocol or on the case report forms. The sponsor will inform the research team which SAEs should be reported to the REC.

The following adverse events are 'expected' (for details see protocol sect. 8.3):

- Perioperative MI
- Cardiac arrest
- Haemodynamic support
- Arrhythmias
- Pulmonary complications
- Thromboembolic complications
- Renal complications
- Infective complications
- GI complications
- Neurological complications
- Bleeding requiring reoperation
- Mediastinitis requiring
- Wound dehiscence requiring rewiring or treatment
- Death

Data on these adverse events collected during the trial will be regularly reported to the trial DMSC for review.

Data on adverse events will be collected from the time of surgery for the duration of the participant's post-operative hospital stay. Data on serious adverse events and follow up data on non-serious adverse drug reactions will be collected from the time of surgery and throughout the 4-6 weeks follow-up period.

Frequencies of these adverse outcomes will be tabulated, in line with guidelines for reporting adverse events in trials.

6.3 Laboratory Data

Any normalising or validating of laboratory values will be carried out before these data are transferred to the CTU.

6.4 Vital Signs, Electrocardiogram and Other Safety Assessments

Vital signs and other safety assessments (e.g. body temperature, pump flow etc.) will be listed individually and summarised by treatment group and in total on each time point.

7 References

Bamnote S (2012). Superior gRaphics in Statistical Reports. Phuse.
http://www.phusewiki.org/wiki/index.php?title=Superior_gRaphics_in_Statistical_Reports

Steiner M E (2015), Effects of Red-Cell Storage Duration on Patients Undergoing Cardiac Surgery, NEJM, 372:15, 9 April 2015, 1419-1429.

Ranieri M V (2012), Acute Respiratory Distress Syndrome, JAMA, 307(23), June 2012, 2526-2533.

Euroqol

http://www.euroqol.org/fileadmin/user_upload/Documenten/PDF/Folders_Flyers/EQ-5D-3L_UserGuide_2015.pdf

8 Appendices

8.1 Publication strategy

It is anticipated that the trial results will form part of a wider publication that will include earlier research carried out on the same theme. This earlier work was conducted in collaboration with the Leicester Cardiovascular BRU. The paper is planned to be submitted to the Journal of Clinical Investigation.

8.2 Roles in the analysis

The trial statistician is Tom Morris. Arne Ring was the supervising statistician until 20th February 2015. Cassey Brookes will take over as supervising statistician from June 2015. The CI is Gavin Murphy.

According to CTU SOPs ST-001/002, the trial statistician has the overall responsibility for planning and implementing the trial analysis and its quality assurance. The supervising statistician will contribute to the planning, implementation and validation of the analysis.

According to UoL SOP S-1030, the Chief Investigator must ensure that it is finalised following review by appropriate personnel and approved by the statistician and Sponsor.

Both statisticians and the CI will review and approve the analysis plan.

All SAP authors will be responsible for review and interpretation of the results and will contribute to any publications that are based on these results.

8.3 Technical specification

The technical specifications will be provided as separate documents.

- The Table of contents outlines the titles of the displays for the statistical report (in EXCEL format).
- The SAP appendix document provides display templates and documents details of the programming of the analysis.
- Details of all the outcome measures are provided as a separate document.