List of abbreviations

Acronym	Details
AE	Adverse event
AKI	Acute kidney injury
AKIN	Acute kidney injury network
ARDS	Acute respiratory distress syndrome
BMI	Body mass index
CABG	Coronary artery bypass graft
CCS	Canadian cardiovascular society
CI	Confidence interval
CICU	Cardiac intensive care unit
CPAP	Continuous positive airway pressure
CPB	Cardiopulmonary bypass
CRF	Case report form
CT	Computed tomography
CVA	Cerebrovascular accident
DOB	Date of birth
eGFR	Estimated glomerular filtration rate
FFP	Fresh frozen plasma
GMR	Geometric mean ratio
HDU	High dependency unit
HR	Hazard ratio
ICU	Intensive care unit
IQR	Inter quartile range
ITT	Intention to treat
IV	Intravenous
LIMA	Left internal mammary artery
LV	Left ventricular
MAR	Missing at random
MD	Mean difference
MI	Myocardial infarction
MRI	Magnetic resonance imaging
NYHA	New York heart association
OR	Odds ratio
PH	Proportional hazards
PIL	Patient information leaflet
PT	Preferred term
RBC	Red blood cell
RCT	Randomised controlled trial
RIMA	Right internal mammary artery

Acronym	Details
RRT	Renal replacement therapy
SAE	Serious adverse event
SAP	Statistical analysis plan
SD	Standard deviation
SIRS	Systemic inflammatory response syndrome
SOC	System organ class
SVT	Supraventricular tachycardia
TIA	Transient ischaemic attack
TR	Time ratio
VF	Ventricular fibrillation
VT	Ventricular tachycardia
WBC	White blood cell

1 INTRODUCTION TO SAP

1.1 Scope

This document details information regarding the statistical analysis of the TITRe2 trial and covers all of the analysis of trial data outlined in the study protocol, with the exception of the health economic analyses.

1.2 Editorial changes

Any changes made to this Statistical Analysis Plan (SAP) after approval must be clearly justified and documented as an amendment at the end of this document. The SAP should then be re-approved.

1.3 SAP document approval

The trial statistician should authorise this document.

2 STUDY BACKGROUND AND OBJECTIVES

2.1 Study background

TITRe2 is a UK wide, multi-centre, open randomised controlled trial (RCT).

Two thresholds for red blood cell (RBC) transfusion following cardiac surgery are compared: a "restrictive" threshold whereby transfusions are given if the haemoglobin (Hb) level is below 7.5g/dL (or haematocrit (Hct) < 22) and a "liberal" threshold whereby transfusions are given if the Hb < 9g/dL (or Hct < 27).

2.2 Study objectives

Objectives of the RCT are to:

A. Estimate the difference in the risk of a post-operative infection or ischaemic event between restrictive and liberal transfusion thresholds.
B. Compare the effects of restrictive and liberal transfusion thresholds with respect to a range of secondary outcomes.
C. Estimate the cost-effectiveness of the restrictive compared to the liberal Hb transfusion threshold and describe this in terms of a cost-effectiveness acceptability curve.

This SAP covers objectives A and B.

2.3 Primary outcome

The primary outcome is a binary composite outcome of any serious infectious or ischaemic event in the first 3 months after randomisation. The qualifying events listed below will be included, along with the manner in which they will be verified:

Infectious events	*Definition / method of verification*
Sepsis during index admission	Defined by the following two conditions, both of which must be satisfied for sepsis to be documented: (a) Antibiotic treatment for suspected infection, ***and*** (b) The presence of SIRS[1] within 24 hours prior to start of antibiotic treatment
Wound infection	ASEPSIS[**1**] score >20. Wounds will be assessed at least once during a participant's hospital stay and details of the ASEPSIS assessment added to the study CRF. A questionnaire will be posted for self-completion, or will be administered by telephone, at 3 months to identify wound infections arising after discharge.[**2**]
Ischaemic events	***Definition / method of verification***
Permanent stroke	Clinical report of brain imaging (computerised tomography (CT) or magnetic resonance imaging (MRI)), in association with new onset focal or generalised neurological deficit (defined as deficit in motor, sensory or co-ordination functions).
Myocardial infarction (MI)	Elevated post-operative peak serum Troponin I or T, verified by an adjudication committee. Further details are given on the following page.
Acute kidney injury (AKI)	AKI Network criteria for AKI, stage 1, 2 or 3 (see below)[**3**] ***Stage 1:*** serum creatinine increase ≥0.3mg/dl (≥ 26.4µmol/l), OR >1.5 and ≤2-fold serum creatinine increase compared to the pre-operative serum creatinine (baseline) value, OR urine output <0.5ml/kg for 6 hours. ***Stage 2:*** >2 and ≤3-fold serum creatinine increase compared to the pre-operative serum creatinine (baseline) value, OR urine output <0.5ml/kg for >12 hours. ***Stage 3:*** >3-fold serum creatinine increase compared to the pre-operative serum creatinine (baseline) value, OR serum creatinine ≥4.0 mg/dl (≥354 µmol/l) with an acute increase of at least 0.5 mg/dl (44 µmol/l), OR urine output <0.3 ml/kg per hour for 24 hours or anuria for 12 hours, OR need for renal replacement therapy (RRT) irrespective of AKI stage at time of RRT. The time of onset of AKI, used to determine whether the event occurred pre-randomisation, is the first time that the patient triggers for AKI regardless of whether this is due to urine output or serum creatinine. The AKI stage recorded is the highest stage reached by the patient post-operatively but pre-

[1] SIRS - systemic inflammatory response syndrome. SIRS is central to the diagnosis of infective complications. It will be defined as ≥2 of the following conditions: temperature >38°C or <36°C; heart rate >90 beats/minute; respiratory rate >20 breaths/min or $PaCO_2$ <32 mm Hg or $PaCO_2$ <4.3 kPa; WBC count >12,000/mm^3 or <4,000/mm^3. Blood test results and temperature will be classified using standard reference ranges.

	discharge.
Gut infarction	Laparotomy or post mortem

Events occurring post-discharge only contribute to the primary outcome if the potentially qualifying event resulted in admission to hospital or death. The exception to this is post-discharge wound infections, which are ascertained using the ASEPSIS post-discharge surveillance assessment. Other suspected infectious events treated in the community that did not cause readmission to hospital will not be recorded because they cannot be validated and are less serious than peri-operative infections.

Events suspected to qualify for the primary outcome but not supported by objective evidence will be referred to an independent adjudication committee whose members will be blinded to the random allocation. In practice this will amount to MIs only, as for all other elements documentary objective evidence has been collated and verified by research nurses blinded to the random allocation at the co-ordinating centre. Therefore the adjudication committee will be required to reach a final decision about whether patients with a suspected MI have actually had an MI, based on patient history, Troponin levels and preoperative and postoperative ECGs. The adjudication committee will consist of three clinical specialists, and agreement between two of the three specialists will be required to reach a final decision.

2.4 Secondary outcomes

Secondary outcomes are listed in the study protocol as:

- Units of RBCs and other blood components transfused during a participant's hospital stay
- Proportion of patients experiencing an infectious event
- Proportion of patients experiencing an ischaemic event
- EQ5D [**4**]
- Duration of intensive care unit (ICU) / high dependency unit (HDU) post-operative stay
- Duration of post-operative hospital stay
- All-cause mortality
- Significant pulmonary morbidity, comprising (i) initiation of non-invasive ventilation (e.g. continuous positive airway pressure (CPAP) ventilation), (ii) re-intubation/ventilation, or (iii) tracheostomy
- Cumulative resource use, cost, and cost-effectiveness

The latter outcome listed above is not covered by this SAP.

2.5 Changes to the study objectives during the course of the study

Some minor changes have been made to the study over the course of the trial:

- A protocol amendment was made to include Troponin T in addition to Troponin I in defining MI, and remove the defined threshold for MI in the study protocol. The highest troponin level for all patients with a suspected MI is collected and a definitive definition of MI will be decided upon after blinded review by the adjudication committee (see section 2.3).
- One of the secondary outcomes (significant pulmonary morbidity) was added part way through the trial.
- In the study protocol one of the intended subgroup analyses is pre-operative renal impairment, defined by pre-operative creatinine ≤177 μmol/l vs creatinine >177 μmol/l. However during the course of the trial use of pre-operative creatinine for risk stratification has been totally superseded by estimated Glomerular Filtration Rate (eGFR). Therefore the subgroup analysis on renal impairment has been amended to eGFR ≤60ml/min vs eGFR >60ml/min (note this has not been covered by a protocol amendment).
- The timings of all primary and secondary outcomes have been clarified as occurring post-randomisation, rather than post-operative.

All required data for the changes/additions were already being collected.

3 STUDY POPULATION

The study population is all adult patients (aged 16 or over) undergoing non-emergency elective cardiac surgery (this includes non-emergency cases admitted from home or non-emergency inpatient cases). Eligibility criteria are as inclusive as possible to promote the applicability of the evidence obtained during the trial.

The planned sample size is 2000 randomised patients. A graph showing recruitment trends over time will be given as well as centre-specific screening data.

3.1 Flow of participants

Participant flow will be described via a flowchart.

3.1.1 Whilst in hospital

Participants consent to the study pre-surgery if they meet all of the pre-consent eligibility criteria and give written consent. They are then randomised if at any point post-surgery they meet the post-consent eligibility criteria (Hb falls below 9g/dL or Hct below 27%). This means that a significant proportion of patients (~45%) consent to the study but are not randomised.

For randomised patients the duration of intervention in the trial is the duration of the patient's care under the consultant cardiac surgeon or a maximum of 3 months after the date of randomisation, whichever is shorter. Almost always, the duration of care under the cardiac surgeon will be the period of hospitalisation after surgery. The majority of data collection is undertaken whilst the participant is an in-patient.

3.1.2 Follow-up

After patients have been discharged from hospital they are followed up at further time points:

- At approximately six weeks post-operatively they are sent an EQ5D questionnaire.
- At approximately three months post-operatively they are contacted by telephone or post to complete a questionnaire including the following elements: a) adverse events (AEs) occurring after discharge; b) questions to identify surgical wound infections occurring after discharge (ASEPSIS post-discharge surveillance questionnaire)[**1**]; c) health economics / resource use questionnaire; d) questions determining whether a participant is aware of his/her random allocation. At this point they are also asked to complete a further EQ5D questionnaire.
- Patients that consent but are not randomised are also sent an EQ5D questionnaire at approximately three months post-operatively.

The duration of follow-up in the trial is until the three month follow-up assessment questionnaires have been completed or until 3 months after randomisation if a participant does not complete the questionnaires.

3.1.3 Follow-up windows

Although the follow-up times are planned at six weeks and three months post-operatively, occasionally data collection is delayed. When this occurs the following rules will be used to determine whether data should be included in analyses:

- EuroQol EQ5D: to determine suitable time frames within which data will be used, the distribution of time between questionnaire completion and operation date will be examined by group, blinded to allocation, separately for each time point. If the

distributions differ between the groups, pre-specified windows will be used: a) for pre-operative questionnaires a window of within 3 months pre-operatively; b) for the 6 week assessment a window of 4-10 weeks after the operation date; c) for the 3 month assessment a window of 10-20 weeks after the operation date. If the distributions are balanced across the two groups, any times that appear to be extreme outliers (identified by eye) will be excluded but all other data collected will be used.

- Three month telephone/postal questionnaire: the questionnaire specifically asks about the three month post-operative period and staff completing the telephone questionnaires are trained to only record information regarding this period, therefore data from all questionnaires will be used. Where dates of events are recorded (this is the case for AEs and the majority of resource use questions), any events inadvertently recorded that occurred more than three months post randomisation will not be included in any analyses. If dates are missing the event will be assumed to have occurred within the three month follow up period.

3.2 Comparisons of patients characteristics

3.2.1 Comparisons of non-consented and consented patients

The only characteristics available for patients that do not consent to the study are age and sex. These characteristics will be described for the following groups of patients:

- Non-consented (including PIL not sent, not approached, ineligible, did not consent and other reason for exclusion from study)
- Consented

This will only be done for sites known to have complete screening data; we anticipate these sites to be Bristol, Southampton and Leicester. Completeness of screening data is ascertained from knowledge about site-specific screening processes, and reflects whether the site screens the majority of patients admitted for cardiac surgery or predominantly those who are considered for inclusion in the trial. Screening log data for Bristol will be supplemented with data from institutional cardiac surgery databases, to identify any patients not recorded on the screening log but who could potentially have been considered for TITRe2. No formal statistical comparisons will be made.

3.2.2 Comparisons of non-randomised (but consented) and randomised patients

Characteristics that will be described include: all pre-operative characteristics, operation type, post-operative Hb/Hct values, blood products transfused, status (alive/dead) at end of surgery and hospital discharge, and EQ5D scores pre-operatively and at 3 months post-operative. These characteristics will be described for the following groups of patients:

- Consented patients considered for randomisation but not randomised (i.e. all consented patients including those randomised in error and excluding: patient/clinician withdrawals pre-surgery, patients who died pre-surgery or surgery was not performed, other reasons not considered for randomisation).
- Randomised patients included in the analysis population (i.e. all randomised patients, excluding: those randomised in error, or patients who withdrew and were unhappy for data collected to be used).

In both groups of patients, the excluded patients do not have the relevant data collected and so cannot be included in the comparisons.

No formal statistical comparisons will be made. Note that comparisons of resource use will be carried out by the health economists and so is not covered in the scope of this SAP.

3.3 Randomisation

Participants are randomised (1:1 allocation) to either the liberal or restrictive group using an internet-based system (Sealed Envelope Ltd). Cohort minimisation is used to minimise imbalance of: a) centre and b) operation type (classified as CABG, Valve, CABG+ Valve and Other).

3.4 Withdrawals

There are two types of study withdrawal, which are documented on a specific case report form (CRF):

- Patient withdrawal: patients can withdraw from the study at any time (including post-consent but prior to randomisation). Reasons for withdrawal are collected along with:
    a) whether data already collected can be used
    b) whether the patient is happy to participate in follow-up
- Clinician decision to discontinue treatment according to protocol: clinicians can decide to discontinue the patient's treatment at any time (this can include post-consent but prior to randomisation, which may happen if a patient's condition changes and the clinician feels decisions about the patient's care should not depend upon the study protocol) . This does not constitute a withdrawal and data collection continues as planned (unless the patient also withdraws their consent) but transfusions are no longer required to be given according to the study protocol.

Withdrawals and treatment discontinuations are summarised by treatment allocation, if applicable.

Unless patients were unhappy for data collected to be used, data on all withdrawals or treatment discontinuations will be included in the study analyses on an intention to treat basis (ITT), see **section 3.6**.

3.5 Protocol deviations

3.5.1 Non-compliance with randomisation protocol

The following types of protocol deviation will be considered:

- Patient did not meet one or more of the pre-consent study eligibility criteria but was consented into the study.
- Patient did not meet the post-consent eligibility criteria (i.e. Hb did not drop below 9g/dL or Hct below 27%) but was randomised.
- Patient was randomised more than 24 hours after meeting the post-consent inclusion criteria (i.e. randomised more than 24 hours after Hb dropping below 9g/dL or Hct below 27%).
- Patient consented and met the post-consent inclusion criteria (i.e. Hb dropped below 9g/dL or Hct dropped below 27%) but was not randomised.

The frequency of each type of protocol deviation will be described.

3.5.2 Non-compliance with transfusion protocol

The following types of protocol deviation will be described:

- Patient received a RBC transfusion outside of protocol.
- Patient was not given a RBC transfusion that, according to the protocol, should have been given.

Such compliance will be assessed for the period from randomisation to hospital discharge. If patients withdraw or have their treatment discontinued, compliance after the time of withdrawal/discontinuation will not be assessed. For both of the above types of non-compliance, instances will be classified into mild, moderate or severe dependent on the likely influence on transfusion rates, and therefore possible influence on study outcomes:

	Transfusion outside of protocol	Transfusion according to protocol withheld
Mild	N/A	A transfusion took place, but more than 24 hours after the relevant breach of the transfusion threshold
Moderate	Patient transfused outside of protocol, but patient did breach the threshold for transfusion at some point post-operatively	Patient was not transfused following a breach, but the patient had previously had at least one post-randomisation transfusion
Severe	Patient transfused outside of protocol, and patient did not breach the threshold for transfusion at any point post-operatively	Patient was not transfused following a breach, and patient had no post-randomisation transfusions

Note it may be possible for patients to be classified as a protocol deviation for more than one reason.

The frequency of each type of deviation will be tabulated by treatment allocation.

Additional analyses will be carried out looking at non-compliance with the transfusion protocol in further detail:

- The following characteristics of different non-compliance will be described by treatment group:
  - Reasons for deviations
  - Number of deviations per patient
  - Hb/Hct levels at deviation
  - Day of week
  - Time of day (weekday, evening or weekend)
  - Time of year (split as Feb-Apr, May-Jul, Aug-Oct and Nov-Jan, to reflect the time of year when changes to junior medical staff are made)

  For withheld transfusions only:
  - Number of previous breaches of transfusion threshold for withheld transfusions
  - Time from first breach of transfusion threshold to transfusion
- Descriptive analyses will be carried out to investigate any differences in patient baseline and operative characteristics between those with and without non-compliance. This will be done separately for any non-compliance and any severe non-compliance, with patient characteristics compared within randomised group.
- The rates of non-compliance with the transfusion protocol across the sites will be described graphically. These will compare the proportions of patients with a) any non-compliance and b) any severe non-compliance with the transfusion protocol.

- Finally, at the beginning of the trial sites were asked to give feedback on standard transfusion protocols to gauge how the trial protocol differed from standard procedures. At the end of the study this exercise will be repeated, with sites being asked additional information on how and when protocols have changed. This information will be summarised as part of the trial reporting.

3.6 Analysis population

The analysis will consist of all randomised patients, excluding:

- Patients marked as "randomised in error": this is a small number of patients (<10) for whom it is realised shortly after randomisation and prior to any intervention that are not eligible
- Patients withdrawn who were unhappy for data collected to be used.

All study analyses will be performed on a modified ITT basis.

3.7 Safety population

Safety data will be analysed on an ITT basis, and will therefore be the same as the analysis population. Note that often safety data are analysed as the treatment received rather than on an ITT basis, however in this study that will not be feasible as protocol deviations do not constitute a "cross-over" between groups. In addition, as the primary outcome is a measure of risk and is analysed on an ITT basis, it will be consistent to also analyse safety data on an ITT basis.

4 DERIVATIONS

4.1 Primary outcome

The primary outcome is defined as follows:

Component event	***Within index admission***	***After hospital discharge***
Sepsis[1]	**YES**, if on CRF C5 there is at least one antibiotic course with: - Date/time antibiotic course started≥date/time of randomisation, AND - *SIRS total*[2]≥2 **NO**, if: - Patient was not given any antibiotics in their post-operative stay (excluding prophylaxis), OR - For all courses of antibiotics, either: o Date/time course started<date/time of	**YES**, if a readmission form (X1) has been completed with: - Infective complication=Yes, AND - Date of admission is within 3 months of operation **NO**, if the above conditions are not met and patient completed 3 month follow-up/died **MISSING**, otherwise

	randomisation, OR ○ *SIRS total*=0 and *SIRS missing*[2]≤1 ○ *SIRS total*=1 and *SIRS missing*=0 **MISSING**, otherwise	
Wound infection[3]	**YES**, If at least one wound with in-hospital asepsis score >20 **NO**, if all scored wounds have in-hospital asepsis score ≤ 20, and no wounds have missing in-hospital asepsis scores **MISSING**, otherwise	**YES**, if at least one wound with post-discharge asepsis score >20 **NO**, if all scored wounds have post-discharge asepsis score ≤ 20, and no wounds have missing post-discharge asepsis scores **MISSING**, otherwise
Permanent stroke[1]	**YES**, if on CRF C6: - Stroke=Yes, AND - Date/time of stroke≥date/time of randomisation, AND - Verified by CT=Yes or verified by MRI=Yes, or verification criteria missing **NO**, if: - Stroke=No, OR - Stroke=Yes and date/time of stroke<date/time of randomisation, OR - Stroke=Yes and verified by CT=No and verified by MRI=No **MISSING**, otherwise	**YES**, if a readmission form (X1) has been completed with: - Stroke=Yes, AND - Date of admission is within 3 months of operation, AND - Verified by CT=Yes or verified by MRI=Yes, or verification criteria missing **NO**, if the above conditions are not met and patient completed 3 month follow-up/died **MISSING**, otherwise
MI	**YES**, if on CRF C6: - Suspected MI=Yes, AND - Date/time of MI≥date/time of randomisation, AND - At least 2 out of 3 adjudication committee members agree that an MI has occurred **NO**, if: - Suspected MI=No, OR - Suspected MI=Yes and date/time of MI<date/time of randomisation, OR - Suspected MI=Yes and at least 2 out of 3 adjudication committee members agree that an MI has not occurred **MISSING**, otherwise	**YES**, if a readmission form (X1) has been completed with: - Suspected MI=Yes, AND - Date of admission is within 3 months of operation, AND - At least 2/3 adjudication committee members agree that an MI has occurred **NO**, if the above conditions are not met and patient completed 3 month follow-up/died **MISSING**, otherwise
AKI[1]	**YES**, if on CRF C6: - AKI=Yes, AND - Date/time of AKI≥date/time of randomisation, AND - Acute Kidney Injury Network (AKIN) criteria stage 1, 2 or 3=Yes or missing	**YES**, if a readmission form (X1) has been completed with: - AKI=Yes, AND - Date of admission is within 3 months of operation, AND

	NO, if: - AKI=No, OR - AKI=Yes and date/time of AKI<date/time of randomisation, OR - AKI=Yes and AKIN criteria stage 1, 2 or 3=No **MISSING**, otherwise	- AKIN criteria stage 1, 2 or 3=Yes or missing **NO**, if the above conditions are not met and patient completed 3 month follow-up/died **MISSING**, otherwise
Gut infarction[1]	**YES**, if on CRF C6: - Gut infarction=Yes, AND - Date/time of gut infarction≥date/time of randomisation, AND - Verified by laparotomy=Yes or verified by post mortem=Yes, or verification criteria missing **NO**, if: - Gut infarction=No, OR - Gut infarction =Yes and date/time of gut infarction <date/time of randomisation, OR - Gut infarction =Yes and verified by laparotomy=No and verified by post mortem=No **MISSING**, otherwise	**YES**, if a readmission form (X1) has been completed with: - Gut infarction=Yes, AND - Date of admission is within 3 months of operation, AND - Verified by laparotomy=Yes or verified by post mortem=Yes, or verification criteria missing **NO**, if the above conditions are not met and patient completed 3 month follow-up/died **MISSING**, otherwise

Notes:

[1] For sepsis, stroke, AKI and gut infarction the event will default to NO if the documentary evidence does not support that the event occurred.

[2]SIRS elements are defined as:

- *Temperature: YES if >38°C or <36°C, NO if 36-38°C, MISSING otherwise*
- *Heart rate: YES if >90 beats/minute, NO if ≤90 beats/minute, MISSING otherwise*
- *Respiration: YES if respiratory rate >20 breaths/min OR $PaCO_2$ <32 mm Hg or <4.3 kPa, NO if respiratory rate ≤20 breaths/min, MISSING otherwise*
- *White blood cell (WBC): YES if >12,000/mm^3 or <4,000/mm^3, NO if 4,000-12,000/mm^3, MISSING otherwise*

***SIRS total** = total of (temperature, heart rate, respiration, WBC), with YES=1, NO=0*

***SIRS missing** = number of missing elements of (temperature, heart rate, respiration, WBC)*

[3] For details of how to derive in-hospital and post-discharge asepsis scores see Supplementary Material.

Separately for pre- and post-discharge, the composite primary outcome is defined as:

- If any of the component events occurred, the composite primary outcome is classified as occurring.
- If all of the component events did not occur (with no missing components), the composite primary outcome is classified as not occurring.

- Otherwise (i.e. there is missing data for at least one of the component events, and all non-missing component events did not occur), the composite primary outcome is classified as missing.

Overall (at any time), the composite primary outcome is defined as:

- If the composite outcome occurred pre-hospital discharge and/or post-discharge, the overall composite outcome is classified as occurring.
- If the composite outcome did not occur either pre- or post-discharge, the overall composite outcome is classified as not occurring.
- Otherwise (i.e. the outcome is missing either pre- and/or post-discharge, and, if applicable, did not occur at the other time point), the composite outcome is classified as missing.

The time to primary outcome occurring is defined as follows:

Situation	**Time to primary outcome defined as**
One or more of the components occur within the index admission	Time (in hours) from randomisation to the onset of the first event (note: the timing of asepsis is assumed to be either: a) the date of sepsis if sepsis is also reported, or b) the halfway point of the participant's post-randomisation stay (i.e. halfway between the randomisation date and the date of discharge from the cardiac surgery unit)
No components occur in the index admission, but one or more occur after discharge	Time (in days) from randomisation to the hospital admission where the event was reported (note: the timing of asepsis will be defined as either a) the date of sepsis if sepsis is also reported, or b) the halfway point between discharge date and 6 weeks post-operatively)
No components occur, patient completed 3 month follow-up	Censored as the time (in days) between randomisation and follow-up
No components occur, patient did not complete 3 month follow-up	Censored as the time (in days) between randomisation and hospital discharge (or death if the patient died prior to 3 month follow-up)

4.2 Secondary outcomes

The following secondary outcomes require derivations to be made:

Secondary outcome	**Rules**
Infectious events	**YES**, if sepsis=Yes, OR wound infection=Yes
	NO, if sepsis=No AND wound infection=No
	MISSING, otherwise
Ischaemic events	**YES**, if stroke=Yes, OR MI=Yes, OR AKI=Yes, OR gut infarction=Yes
	NO, if stroke=No, AND MI=No, AND AKI=No, AND gut infarction=No
	MISSING, otherwise

Secondary outcome	Rules
RBC units transfused intra-operatively	Total number of units listed on CRF B2 with reason for transfusion=A[2] (intra-operative)
RBC units transfused during pre-randomisation re-operation	Total number of units listed on CRF B2 with reason for transfusion=B (re-operation) and date/time < date/time of randomisation
RBC units transfused during post-randomisation re-operation	Total number of units listed on CRF B2 with reason for transfusion=B (re-operation) and date/time ≥ date/time of randomisation
RBC units transfused after treatment according to protocol discontinued	Total number of units listed on CRF B2 with reason for transfusion=C (treatment according to protocol discontinued)
RBC units transfused post-operative but pre-randomisation	Total number of units listed on CRF B2 with reason for transfusion=D (pre-randomisation)
RBC units transfused in breach of protocol	Total number of units listed on CRF B2 with reason for transfusion=E (in breach of protocol)
RBC units transfused per protocol	Total number of units listed on CRF B2 with reason for transfusion=F (per protocol)
Total RBC units transfused	Total number of RBC units listed on CRF B2
Total duration of post randomisation ICU/HDU stay (hours)	Calculated as the sum of the following components: – Duration of initial cardiac intensive care unit (CICU)/HDU stay = Earliest of (ward admission date/time, general ICU date/time, discharge date) – Latest of (Randomisation date/time, CICU/HDU admission date/time) * 24 – Duration of initial general ICU stay (if applicable) = (Date/time of next admission following general ICU admission) – Latest of (Randomisation date/time, Date/time of general ICU admission) * 24 – Duration of any readmissions to CICU/HDU/general ICU: (Date/time of next admission following relevant readmission) – Latest of (Randomisation date/time, Date/time of CICU/HDU/general ICU readmission) * 24
ICU/HDU censor variable	**YES** if patient died during ICU/HDU stay **NO** otherwise
Duration of post randomisation hospital stay	(Date of discharge from cardiac surgery unit or date of death) – (Randomisation date)
Postoperative hospital stay censor variable	**YES** if patient died during hospital stay **NO** otherwise

[2] Note: for early versions of the study CRFs, reasons for transfusions were not recorded and therefore will be derived from dates/times of transfusions, operation, re-operation, treatment discontinuation, randomisation and Hb/Hct levels at transfusions.

Secondary outcome	Rules
All-cause mortality	**YES**, if either: − Patient recorded as dead at discharge from hospital on CRF B1 and/or D1 − A SAE form (CRF F1) has been completed with either: reason for reporting SAE=patient died, OR outcome of SAE=death AND date of death is within 3 months of operation date − NHS mortality tracing shows the patient died with a date of death within 3 months of operation date **NO**, otherwise
Time to death (days)	(Date of death – Randomisation date)
Significant pulmonary morbidity	**YES**, if: EITHER, on CRF C7 any of the following are true: − Initiation of mask CPAP=Yes AND date/time ≥date/time of randomisation, OR − Re-intubation/ventilation=Yes AND date/time ≥date/time of randomisation, OR − Tracheostomy=Yes AND date/time is after date/time of randomisation OR, a readmission form (X1) has been completed with date of admission within 3 months of operation and any of the following are true: − Initiation of mask CPAP=Yes, OR − Re-intubation/ventilation=Yes, OR − Tracheostomy=Yes **NO**, if: On CRF C7: − Initiation of mask CPAP=No OR (Initiation of mask CPAP=Yes AND date/time <date/time of randomisation), AND − Reintubation/ventilation=No OR (Reintubation/ventilation=Yes AND date/time <date/time of randomisation), AND − Tracheostomy=No OR (Tracheostomy =Yes AND date/time <date/time of randomisation) AND, patient completed 3 month follow-up/died and there is not a readmission form completed for initiation of mask CPAP, reintubation/ventilation or tracheostomy **MISSING**, otherwise
EQ5D single summary index score	Five digit 'state' score is derived as: 10000*mobility score + 1000*self-care score + 100*usual activities score + 10*pain/discomfort score + anxiety/depression score. Each state score is then assigned a single summary index score according to reference scales. These index scores are numerical and range from -0.59 to 1.00, with a score of 1.00 denoting perfect health.

4.3 Protocol compliance

New variable	Rules
Did not meet pre-consent	**YES**, if consent=Yes but one or more of the eligibility criteria are not met

New variable	Rules
eligibility criteria but was consented	**NO**, otherwise
Did not meet post-consent eligibility criteria but was randomised	**YES**, if randomisation date non-missing but Hb ≥9g/dL (Hct ≥27%) on all days post-operatively **NO**, otherwise
Randomised >24 hrs after meeting post-con sent eligibility criteria	**YES**, if either: - First day that Hb <9g/dL (or Hct <27%) is 2 or more days before date of randomisation, OR - (Randomisation date/time – Date/time threshold first breached) >1 day **NO**, otherwise
Consented and met post-consent eligibility criteria but not randomised	**YES**, if randomisation date missing but there is at least one day postoperatively when Hb <9g/dL (or Hct <27%), and patient is not withdrawn/treatment discontinued at time Hb <9g/dL **NO**, otherwise

Any transfusion outside of protocol	Transfusions listed on CRF B2 prior to treatment discontinuation/patient withdrawal where one of the following is true:
	- Reason for transfusion=E (outside of protocol)
	- Reason for transfusion=F (per protocol) or missing and:
	  o CRF E1 (reason for giving transfusion outside of protocol) has been completed, OR
	  o Recorded Hb/Hct is above the relevant (treatment group specific) threshold, OR
	  o Transfusion is within 2 hours of a previous transfusion that had the same Hb/Hct (or missing). These are two units of blood given together without rechecking Hb/Hct

Moderate transfusion outside of protocol	Transfusion outside of protocol (i.e. identified from above) whereby patient breached relevant threshold for transfusion at some point post-randomisation
Severe transfusion outside of protocol	Transfusion outside of protocol (i.e. identified from above) whereby patient did not breach the relevant threshold for transfusion at any point post-randomisation
Mild withheld transfusion	Any instances that are more than 24 hours before the next per protocol transfusion whereby: - A CRF E2 (withheld transfusion) was completed - According to CRF B1 the patient breached the relevant threshold on a day that was prior to the breach date for the next per-protocol transfusion, and no CRF E2 was completed or other type of transfusion given on that day - According to CRF B2 the "number of breaches prior to trigger breach" is greater than 0, and these breaches have not been accounted for in the previous two steps - According to CRF B2 the "number of breaches prior to trigger breach" is 0, no CRF E2s have been completed and the answer to the question "Was RBC prescribed within 24h of breach" is No

New variable	Rules
Moderate withheld transfusion	Any instances between the last per protocol transfusion received and discharge whereby the patient breached the relevant threshold for transfusion (identified either via a completed CRF E2 or via a breach on CRF B1).
Severe withheld transfusion	For patients who did not have any post-randomisation transfusions (including post withdrawal, in breach of protocol and per-protocol), any instances post-randomisation whereby the patient breached the relevant threshold for transfusion (identified either via a completed CRF E2 or via a breach on CRF B1).
Any withheld transfusion	**YES**, if mild withheld transfusion=Yes, OR moderate withheld transfusion=Yes, OR severe withheld transfusion=Yes **NO**, otherwise
Any severe protocol deviation (transfusion protocol)	**YES**, if severe extra transfusion=Yes, OR severe withheld transfusion=Yes **NO**, otherwise
Any protocol deviation (transfusion protocol)	**YES**, if any extra transfusion=Yes, OR any withheld transfusion=Yes **NO**, otherwise
Threshold breaches that do not constitute a protocol deviation	Any instances where a CRF E2 has been completed that are within the 24 hour period prior to a per-protocol transfusion

4.4 Other variables

New variable	Rules
Reason for exclusion from study	Exclusion group defined as: - PIL not sent: PIL sent=No, Approach is not Yes, Consent is not Yes - Not approached: PIL sent=Yes, Approach=No, Consent is not Yes - Ineligible: Eligible=No, Consent is not Yes - Eligible but did not consent: Eligible=Yes, Consent=No
Age at randomisation	(Randomisation date – date of birth (DOB))/365.25
Body mass index (BMI)	Weight (kg) / Height $(cm)^2$ * 10,000
EuroSCORE	For all patients start with Euroscore of zero and add points according to the following rules: - Age: <60=0, 60-64=1, 65-69=2, 70-74=3, 75-79=4, 80-84=5, 85-90=6, >90=7 - Sex: Male=0, Female=1 - Chronic pulmonary disease: add 1 - Extracardiac arteriopathy, neurological dysfunction, Creatinine >200 µmol/l, unstable angina, pulmonary hypertension, recent MI, surgery other than isolated CABG: add 2 for each - Previous cardiac surgery, active endocarditis, critical preoperative state, surgery on thoracic aorta: add 3 for each

New variable	Rules
	- Postinfarct septal rupture: add 4 - LV function: Good (>50%)=0, Mod (30-50%)=1, Poor (<30%)=3
Day of randomisation (days post-op)	(Randomisation date – Operation date)
Time between surgery and randomisation (hours)	(Randomisation date/time - Operation date/time) * 24
Day of withdrawal post-op for pre-randomisation withdrawals	(Withdrawal date – Operation date)
Day of withdrawal post-randomisation for post-randomisation withdrawals	(Withdrawal date – Randomisation date)
Day of treatment discontinuation (days post-randomisation)	(Treatment discontinuation date – Randomisation date)
Duration of operation (hours)	(Operation end time – Operation start time) * 24
Complication (on C7) occurred pre-randomisation	**YES** if complication (C7) occurred and date/time of onset<date/time of randomisation **NO** otherwise
Complication (on C7) occurred post-randomisation	**YES** if complication (C7) occurred and date/time of onset≥date/time of randomisation **NO** otherwise
Ventilation time (hours)	Calculated as the sum of the following components: - (Extubation date/time – Randomisation date/time) * 24 - (Re-extubation date/time) – Latest of (Randomisation date/time, re-intubation date/time) * 24 (if applicable)
Duration of ward stay (hours)	Calculated as the sum of the following components: - Duration of initial ward stay = Earliest of (Date/time of next admission following ward admission) – Latest of (Randomisation date/time, Ward admission date/time) * 24 - Duration of any readmissions to ward: (Date/time of next admission following ward readmission) – Latest of (Randomisation date/time, Date/time of ward readmission) * 24
Ward censor variable	**YES** if patient died during ward stay **NO** otherwise
Timing of unexpected SAE	Pre-discharge if SAE start date ≤ discharge date Post-discharge if SAE start date > discharge date
Maximum intensity of unexpected SAE	Maximum of intensity variable on initial SAE form and all follow-up SAE forms
Final outcome of unexpected SAE	Outcome (resolved without sequelae, resolved with sequelae, ongoing, died) according to last SAE form completed (may be initial report or follow-up)

New variable	Rules
Percentage decline in Hb	(Pre-operative Hb (CRF A2) – minimum Hb post-operatively (B1)) / Pre-operative Hb * 100
eGFR	([140–age] * Weight (A2) * [0.85 if female]) / (Pre-op creatinine (mg/dl) * 72)

5 STATISTICAL ANALYSES

5.1 Descriptive data

Baseline (i.e. patient demography and past history) and intra-operative characteristics will be described by treatment group for patients in the analysis population. In addition post-operative outcomes that are not study outcomes or AEs will be described by treatment group.

Continuous variables will be summarised using the mean and standard deviation (SD) (or median and inter quartile range (IQR) if the distribution is skewed), and categorical data will be summarised as a number and percentage. The summary statistic headings given are those we expect to use based on a-priori knowledge of the clinical measurements gained from previous studies. However, if distributional assumptions are not valid, changes will be made.

Any imbalances in the characteristics of the patients will be described but statistical tests for imbalance will not be carried out.

5.2 Primary and secondary outcome data

All outcomes listed in the study protocol will be presented as follows:

5.2.1 Primary outcome

The primary outcome will be summarised as follows:

- The numbers and percentages of patients experiencing at least one element of the primary outcome at any time post-randomisation will be presented by treatment group. This outcome will be analysed as a binary outcome, see section 5.3.2.
- In addition, the numbers and percentages of patients experiencing: a) any infectious event, b) any ischaemic event and c) each of the individual primary outcome components will be given by treatment group.
- The frequency of each combination of component events will be described by deriving a 6-digit variable where each digit relates to one of the components, and takes the value "1" if the patient experienced the outcome, "0" if they did not and "." if the component

is missing. The numbers of patients with each value of this variable will be described by treatment group.

- The time from randomisation to the first occurrence of the primary outcome will also be analysed as a time to event outcome as a secondary analysis (see section 5.3.2). Patients that don't experience the primary outcome will be censored at either:
  - Date of 3 month follow-up, for patients with 3 month follow-up completed
  - Date of death, for patients who die prior to 3 month follow-up.
  - Date of discharge from hospital, for patients who survive 3 months post-operatively but do not complete the follow-up questionnaire.
- Various sensitivity analyses will also be undertaken (see **Section 5.5**).

5.2.2 Secondary outcome: units of RBCs and other blood components transfused during a participant's hospital stay

RBC transfusions

All RBCs transfused post-randomisation will be summarised by the median and IQR (or mean and SD if the data is not skewed, which is unlikely) number of units transfused in each treatment group. This outcome will be analysed as a continuous outcome (see section 5.3.2).

In addition, a more detailed breakdown of the numbers of units transfused will be presented, and the above summary statistics will also be presented split into the four types of transfusion (re-operation transfusions, transfusions after treatment according to protocol has been discontinued, transfusions in breach of protocol, per protocol transfusions). However, no further comparisons between the groups will be made. The total RBC units transfused (both pre- and post-randomisation) will also be given, but no formal comparison made.

Fresh frozen plasma (FFP), platelets and cryoprecipitate transfusions

FFP, platelets and cryoprecipitate transfusions will be summarised by the median and IQR number of units transfused during a participant's hospital stay for each group. All three outcomes will be analysed as continuous outcomes (see section 5.3.2). Note it is not possible to spilt such transfusions into pre- and post-randomisation due to how the data was collected. The numbers of units of RBC, FFP, platelets and cryoprecipate transfused will also be described graphically.

Use of Activated Factor VII and Beriplex

Activated Factor VII and Beriplex use will be summarised by the numbers and percentages of patients in each group for whom the blood product was used. Both outcomes will be

analysed as binary outcomes (see section 5.3.2). Note it is not possible to spilt such product use into pre- and post-randomisation due to how the data was collected.

Hb/Hct levels

The average nadir daily Hb and Hct levels in each group at each day post-randomisation will be described graphically by the mean and SD (or median and IQR if distributions are skewed) in each treatment group. Although no formal comparisons will be made, the Hb/Hct levels at day three post-randomisation (chosen because at this point the differing transfusions regimens will be likely to have had an effect on Hb/Hct levels, and most patients will still be in hospital and have readings available for comparison) in each group will be used as an overall summary measure.

5.2.3 Secondary outcome: proportion of patients experiencing an infectious/ischaemic event

The presentation of the proportion of patients experiencing infectious/ischaemic events is covered within the primary outcome table. Both outcomes will be analysed separately as binary outcomes (see section 5.3.2).

5.2.4 Secondary outcomes: other clinical outcomes

For the presentation of other clinical outcomes (duration of post-operative ICU/HDU and hospital stay, all-cause mortality and significant pulmonary morbidity).

The duration of post-randomisation ICU/HDU and hospital stay, and the time to death (all-cause mortality) will be summarised by the median and IQR in each treatment group. All outcomes will be analysed as time to event outcomes (see section 5.3.2), with censor variables as defined below:

Outcome	Censor variable
Duration of post-randomisation ICU/HDU stay	Time of death in ICU/HDU
Duration of post-operative hospital stay	Time of death in hospital
All-cause mortality	Time of last follow-up (usually 3 months post-operation)

Significant pulmonary morbidity will be summarised as the numbers and percentages of patients in each treatment group experiencing the event. The outcome will be analysed as a binary outcome (see section 5.3.2).

5.2.5 Secondary outcome: EQ5D

The responses to each of the five EQ5D questions (mobility, self-care, usual activities, pain/discomfort and anxiety/depression) will be summarised as the numbers and percentages of patients in each treatment group choosing each response, at each time-point

(pre-operative, 6 weeks post-operative and 3 months post-operative). No formal statistical comparisons between the groups will be made.

The two continuous EQ5D outcomes (single summary index and visual analogue scale) will be summarised as means and SDs (or medians and IQRs if distributions are skewed) at each time point, in each treatment group. Both outcomes will be analysed as continuous longitudinal outcomes, see section 5.3.2.

Note that a summary figure will be produced summarising all the results from sections 5.2.4 and 5.2.5.

5.3 Analysis models

5.3.1 Adjustment in models

The intention is to adjust all models for factors included in the cohort minimisation: operation type (coronary artery bypass graft (CABG) only, Valve only, CABG and valve, Other – with CABG only as the reference group) as a fixed effect and centre as a random effect (or a shared frailty term in time to event models). Occasionally operation type differs between the study database and the randomisation system as it has been entered incorrectly into the randomisation system. In this case the value from the study database will be used, as the operation type recorded on the database has been confirmed to be correct in such instances.

5.3.2 Models for different data types

General methods of assessing treatment effects are outlined below. For all treatment comparisons the liberal group will be the reference group. Details specific to each outcome are described as appropriate.

- **Binary outcomes** (primary outcome, proportions of infectious/ischaemic events, use of Activated Factor VII/Beriplex and significant pulmonary morbidity) will be compared between treatment groups using logistic regression. Formal statistical comparisons of treatment effects will only be performed if more than ten patients in total experience the outcome (with at least one event in each treatment group). Treatment comparison estimates will be presented as adjusted odds ratios (OR) and 95% confidence intervals (95% CI).
- **Continuous outcomes** (units of RBCs, FFP, platelets and cryoprecipitate transfused) will be compared using linear regression. For untransformed data treatment comparisons will be presented as adjusted differences in means with 95% CI, and for logarithmically transformed data as adjusted ratios of geometric means with 95% CI. If

a logarithmic transformation is not satisfactory other analysis/presentation methods will be sought.

- **Time to event outcomes** (duration of ICU/HDU stay, duration of post-operative hospital stay and all-cause mortality) will be compared using Cox's proportional hazards (PH) models, with treatment comparisons presented as hazard ratios (HR) and 95% CI. Such models require an assumption of PH to be met. If such outcomes consist of more than one distinct time periods (e.g. the patient had two separate admission periods in ICU, or the patient was admitted to ICU after they were randomised) time periods may be "split" (e.g. by using the "stsplit" command in Stata) to account for this. Any patients with a time of zero will be included in analyses by assuming a time of half of the smallest non-zero time to event.
- **Continuous longitudinal outcomes** (EQ5D single summary index and visual analogue scale scores) will be compared using linear mixed effects methodology with the treatment group and study design variables (see section 5.3.2) fitted as fixed effects, and patient terms as random effects. Separate parameter estimates will be incorporated into models for 1) the mean baseline response across both treatment groups and 2) at each post-intervention time point for each treatment (i.e. saturated model with time fitted as a categorical variable). This approach of "jointly" modelling the baseline and post-intervention measurements avoids the necessity to either exclude cases with missing baseline measures or to impute missing baseline values. If the time x treatment interaction (post-intervention) is not statistically significant at the 10% level an overall treatment effect will be reported. If the interaction is statistically significant the changes in treatment effect with time will be described. Different variance/covariance structures will be explored, and the structure that provides the best fit in terms of information criteria such as AIC, BIC and likelihood ratio tests will be used. Treatment comparisons will be presented as adjusted differences in means with 95% CI.

5.3.3 Statistical significance

For hypothesis tests two-tailed p-values<0.05 are considered statistically significant. Likelihood ratio tests will be used in preference to Wald tests for hypothesis testing.

5.3.4 Model assumptions

For all methods outlined underlying assumptions will be checked using standard methods, e.g. residual plots, tests for PH, etc. If assumptions are not valid then alternative methods of analysis will be sought. If outlying observations are found which mean models do not fit the data adequately, such observations will be excluded from the main analyses and

comments made in footnotes. Sensitivity analyses may be performed to examine the effect on the study's conclusions of excluding outlying observations.

If there are any boundary problems for either of the EQ5D continuous scales (i.e. if there are an inflated number of patients scoring "perfect health") then alternative analysis methods will be sought. Examples include: creating a binary endpoint from the continuous outcome and analysing using the methods outlined above.

5.3.5 Multiple testing

No formal adjustment will be made for multiple testing. However, the following measures to try and avoid problems with over-interpretation will be taken: 1) formal statistical comparisons will not be made for outcomes with low event rates, and 2) only pre-specified subgroup analyses will be performed (see section 5.4), and a significance level of 5% will be used for the tests for interaction for subgroup analyses despite being low powered tests. Consideration will be taken in interpretation of results to reflect the number of statistical tests performed and the consistency, magnitude and direction of treatment estimates for different outcomes.

5.4 Subgroup analyses

There are seven pre-specified subgroup analyses stated in the study protocol:

- Operation type (isolated CABG **vs** other operation types)
- Age at operation (<75 years **vs** ≥75 years)
- Pre-operative diagnosis of diabetes (none **vs** diet, oral medication or insulin controlled)
- Pre-operative diagnosis of lung disease (none **vs** chronic pulmonary disease or asthma)
- Pre-operative renal impairment (eGFR ≤60ml/min **vs** eGFR >60ml/min)
- Sex (males **vs** females)
- Pre-operative ventricular function (good **vs** moderate or poor)

Each subgroup analysis will be performed by adding a relevant interaction term to the primary outcome logistic regression model (e.g. for sex, a sex*treatment interaction term will be added to the model). The hypothesis for all subgroup analyses is that there will be no interaction. Results of the subgroup analyses will be presented in forest plots, (one for subgroup analyses with statistically significant tests for interaction, and one for those without significant interactions), with ORs and 95% CIs within each subgroup displayed alongside p-values from results of tests for interactions. P-values for treatment estimates within each subgroup will not be given.

Note that for each of the subgroup analyses the first group listed in each set of brackets above will be the reference group (e.g. for age it will be the <75 years group). Also, for the

operation type subgroup analysis, operation type as a four-level variable will not be adjusted for as a fixed effect (see section 5.3.1). No further subgroup analyses will be performed.

5.5 Sensitivity analyses

The following sensitivity analyses have been identified, note these were not pre-specified in the study protocol:

- *Examining treatment estimates for the primary outcome by site, ordering sites by rates of severe non-compliance with the transfusion protocol.* This will be implemented by producing a forest plot of treatment estimates for each site, with the sites ordered by their rates of severe non-compliance with the transfusion protocol. The hypothesis is that the treatment effect should tend towards the null with increasing non-compliance. Any sites with no patients that experienced the primary outcome will be excluded from this analysis, although a footnote will be added indicating the severe non-compliance rates for such sites.
- *Assessing the effect of the timing of randomisation and transfusions on the primary outcome.* This will be implemented by two sensitivity analyses that re-analyse the primary outcome:
  - Excluding all events that occurred in the first 24 hours after randomisation. The justification for doing this is that such events occurring in the first 24 hours after randomisation are less likely to be attributable to the treatment regimen.
  - Excluding patients who were transfused prior to randomisation (either: intra-operative, post-operative but pre-randomisation or during pre-randomisation re-operations). The justification is that it may be these transfusions that lead to the primary outcome rather than any post-randomisation transfusions.
- *Assessing the effect of AKI.* In collecting AKI data, it was unfortunately overlooked that the creatinine rise required to trigger AKI should occur in a 48 hour period. However highest daily creatinine levels have been recorded separately, so the following sensitivity analyses have been planned that re-analyse the primary outcome:
  - Excluding patients identified with AKI who do not have an increase in creatinine over a 48 hour period or less, according to the daily highest creatinine levels collected (accepting that these patients may have triggered AKI anyway due to urine output or renal replacement therapy).

- Including patients that have not been identified as having AKI, but according to their daily highest creatinine levels have a rise in creatinine that would meet the criteria (and were not having haemofiltration or dialysis pre-operatively).

- *Serious primary outcome events.* The interim analysis showed that the majority of the primary outcome events are either sepsis or AKI. Therefore the primary outcome will be re-analysed including only the more "serious" events. This will mean the following changes to the definitions of the primary outcome:
  - All MIs, gut infarctions and strokes will be included
  - Only AKI stage 3 events will be included
  - All asepsis events will be excluded (the more serious wound infections will be identified via serious sepsis events)
  - For pre-discharge sepsis events: serious events will be identified via presence of sepsis plus organ failure (defined as: MI, stroke, AKI, laparotomy for gut infarction and one or more of reintubation, ARDS, low cardiac output and/or tracheostomy; for these latter events the event must meet the criteria of an SAE).
  - Post-discharge sepsis events will be included (as they require hospitalisation)

5.6 Pre-specified observational analyses

There are three pre-specified observational analyses in the study protocol:

1. Estimating the relationship between the number of RBC units transfused, and the risk of mortality and morbidity, stratified by trial arm.
2. Investigating the relationship between percentage decline in Hb from the preoperative level and the risk of primary and secondary outcomes, taking into account the number of RBC units transfused.
3. Investigating whether RBC age is associated with the risk of primary and secondary outcomes.

Planned tables and figures for these analyses are not included in this SAP, as they are likely to vary dependent upon the final models used. However, in brief, tables of pre-operative and intra-operative characteristics will be presented by the (categorised) exposure of interest as well as tables reflecting the models fitted and variables adjusted for.

Some preliminary analysis techniques are outlined below; however the final techniques used are likely to change dependent upon the findings of a) exploratory analyses, and b) the analysis of the trial primary and secondary outcomes. This section of the SAP may therefore be reviewed and expanded once the main trial outcomes have been analysed.

5.6.1 Analysis of number of RBC units transfused and percentage decline in Hb (analyses 1 and 2)

Analyses 1 and 2 will be implemented by fitting logistic regression models, with an outcome of the primary outcome and/or all-cause mortality. Three separate models will be fitted to address the hypotheses posed by both analyses, with the following explanatory variables:

- Model 1: total number of RBC units transfused (either pre- or post-randomisation)
- Model 2: percentage decline in Hb
- Model 3: total number of RBC units transfused and percentage decline in Hb

In all of these models the following variables will be adjusted for if found to be potential confounders: randomised allocation, operation type, centre (as a random effect), EuroSCORE, age and sex.

Points of note:

- The total number of RBC units transfused will be fitted as either a continuous variable or an ordinal categorical variable, dependent upon model fit.
- The percentage decline in Hb will be defined as the percentage change from the pre-operative value to the lowest Hb level reached post-operatively and prior to the primary outcome.

5.6.2 Age of blood analysis (analysis 3)

Analysis 3 will be achieved by linking the batch numbers of all RBCs transfused to a blood bank database. The age of each unit transfused will then be determined from the date of donation and date of transfusion. A logistic regression model will be fitted with an outcome of primary outcome and/or all-cause mortality as the outcome variable and the age of blood as the exposure.

For the primary analysis age of blood will be defined as the age of the 'oldest' unit of blood transfused at any time (i.e. including intra-operative, during re-operations, pre-randomisation and post-randomisation). The following variables will be adjusted for if found to be potential confounders: number of RBC units transfused, blood group, EuroSCORE, age and sex.

Points of note:

- The sensitivity of fitting the model using the age of the 'oldest' unit of blood will be explored by refitting the model using other definitions of the exposure variable. This may include: the mean age of all RBC units, the use of any blood more than 14 days old, the number or percentage of RBC units given that are more than 14 days old, the

use of blood that is older than the median age of all RBC units transfused. There are known problems with all of these approaches, e.g.: the age of the 'oldest' unit of blood is likely to be confounded by the number of RBC units transfused, the use of any blood more than 14 days old is likely to be confounded by blood group and many of these methods will need to exclude patients not transfused any RBC units.

5.6.3 Points relevant to all analyses:

- Potential confounders are defined as: variables associated with both the exposure and the outcome that are not an intermediary step on the causal pathway between the exposure and outcome, that significantly contribute to the relevant multivariate model (defined as a likelihood ratio p-value <0.05 or by modifying the effect estimate by greater than 10%).
- It may be sensible to restrict the analyses to only patients who did not receive a proportionately large number of RBC units (e.g. restrict to those who received five or less units).
- The instrumental variable method of controlling for confounding will be explored.
- In all of the analyses (with the exception of decline in Hb) there is a potential problem that some of the RBCs may be transfused after the outcome. Therefore fitting the models described above may not be appropriate due to the timing of the exposure relative to the outcome. If this proves to be the case then alternative approaches will be considered, including:
  - Nested matched case-control study: each patient with the primary outcome (i.e. case) will be matched to a control (by matching on at least centre and randomised allocation, other factors may also be used). For both the case and the control any RBC units transfused after the time that the case first experienced the primary outcome will be excluded from analyses.
  - Time to event analyses with a time varying covariate of RBC units given: this would address the issue of exposure time (for cases the event would be the primary outcome event, and for controls the last follow-up), but would ignore any blood given after the occurrence of an outcome event.

5.7 Meta-analysis combining the results of TITRe2 with other studies

It is intended to perform a meta-analysis combining the primary outcome results from this study with any previous systematic reviews and studies. This analysis will be performed using standard meta-analysis methods for binary outcomes, using a random effects model. Results will be presented in a forest plot.

Previous studies will be included in the meta-analysis if they fulfil the following criteria:

- The patient population was patients undergoing cardiac surgery.
- Restrictive and liberal RBC transfusion strategies are compared, although the actual Hb/Hct thresholds for transfusion can differ between studies.
- The outcomes included in the meta-analysis are post-operative morbidity or mortality – if possible (i.e. there are sufficient numbers of studies) each component of the TITRe2 primary outcome will be analysed separately.

Data from studies will be used individually if possible, i.e. aggregate data from previous systematic reviews/meta-analyses will only be used if individual study level data is not available. Also care will be taken to ensure that data from studies are included only once, i.e. data should not be included as part of a systematic review and then also from the study in its own right.

5.8 Post-hoc analyses

A secondary post-hoc analysis of severe in hospital events will be performed. This will involve refitting the primary outcome model with an outcome of: death, severe sepsis (as defined in section 5.5), ARDS, tracheostomy, low cardiac output, MI, AKI stage 3, gut infarction and/or stroke.

5.9 Missing data

In all tables missing data will be indicated by footnotes. If the amount of missing data differs substantially between treatment groups potential reasons will be explored.

5.9.1 Missing predictor data

There will be no missing data for any of the randomisation factors (by design). All other potential predictors are preoperative measurements of continuous longitudinal outcomes, and due to the joint modelling approach described previously the handling of missing values for such data is considered in the context of missing longitudinal data (see below).

5.9.2 Missing continuous outcome data measured at one time point

- If the proportion of missing data is less than 5% then complete case analysis will be performed (i.e. excluding cases with missing data).
- If the proportion of missing data is between 5% and 15%, marginal mean imputation will be performed, i.e. imputing the overall median or mean for continuous data, or the most common category for binary or categorical data.
- If the proportion of missing data is between 15% and 25% conditional mean imputation methods will be used. This involves predicting the outcome from a regression model

from (linearly related) covariate(s). These covariates will include the design variables, plus other potentially important covariates (e.g. age, gender, additive EuroSCORE).

- If the proportion of missing data is above 25% multiple imputation methods will be considered. A general imputation model that uses an iterative procedure to generate imputed values will be used to generate multiple complete data sets (e.g. using Stata's mi impute). The model of interest will be the fitted to each of the complete data sets and effect estimates combined using Rubin's rules.

5.9.3 Missing longitudinal continuous outcome data

For continuous data measured at multiple time points preoperative values will be modelled jointly with those measured postoperatively, as described previously, thereby allowing all cases with at least one observation to be included. If appropriate (the level of missingness is >20%) then any variables that are predictive of missingness will be identified, and if there is reason to suggest that an assumption of missing at random (MAR) given these variables is reasonable (especially likely if the variable was measured pre-operatively) then such variables will be adjusted for in the models of interest. These models can be shown to provide unbiased estimates of the treatment effect and moreover multiple imputation approaches would not be expected to recover any additional information.

5.9.4 Missing binary or categorical outcome data

No formal imputation techniques will be used for missing binary or categorical outcome data. The following approach will be followed for handling missing data will be used for the primary outcome:

- The amounts of missing data in each treatment group will be described.
- The primary outcome element expected to have the highest amount of missing data is wound infection (asepsis scoring). If in-hospital asepsis scores are missing and the following are true the patient will be assumed to have no wound infection: 1) no antibiotics for suspected wound infection were prescribed in hospital, 2) follow-up is complete and the patient reported no problems with the healing of the wound at follow-up.
- If after the above point has been implemented the level of missing data is greater than 5%, this is likely to be mainly due to missing follow-up data. In this case separate treatment estimates will be made for: 1) primary outcome at hospital discharge, and 2) primary outcome at any time.
- Finally a sensitivity analysis will be carried out reanalysing the primary outcome twice: firstly assuming patients with missing data didn't have the primary outcome and

secondly assuming patients with missing data had the primary outcome. Any impact on treatment difference estimates will be noted.

5.10 Safety data

AEs occurring in the study period for all patients in the safety population will be tabulated. No formal comparisons between treatment groups will be made.

Tables will summarise *expected* AEs listed in the study protocol. Events occurring prior to hospital discharge will be summarised, with events that meet the serious criteria[3] indicated (serious adverse events, SAEs). Such events are captured via the study CRFs. After hospital discharge, only SAEs are collected and will be summarised. Finally the numbers of SAEs occurring at any time will be described (i.e. either pre or post hospital discharge).

Further tables will summarise *unexpected* SAEs, i.e. events that are not listed in the study protocol that meet the serious criteria. Such events are captured via separate SAE report forms and the event type will be coded using the Medical Dictionary for Regulatory Activities (MedDRA). System organ class (SOC) terms will be used to group events, with groupings further broken down into preferred terms (PTs) if necessary.

A summary table of expected and unexpected events combined occurring at any time will also be produced.

5.11 Use of Hb/Hct

At most sites, Hct measurements are not used in treatment decisions. However at approximately a quarter of sites both Hb and Hct measurements are used; e.g. a patient in the liberal group would be transfused if their Hb fell below 9g/dL OR their Hct fell below 27%.

In the presentation of the study results, Hb values are presented unless either: a) Hb is missing and Hct non-missing, b) the Hct is lower than the Hb. In either of these cases the Hct is converted to Hb (by dividing by three) and used in its place.

6 BIBLIOGRAPHY

1. Wilson AP, Treasure T, Sturridge MF, Gruneberg RN. A scoring method (ASEPSIS) for postoperative wound infections for use in clinical trials of antibiotic prophylaxis. *Lancet* 1986;**1**:311-3.

[3] An event is classified as serious if it meets one or more of the following criteria: a) resulted in death, b) was life threatening, c) resulted in persistent or significant disability/incapacity, d) prolonged an ongoing hospitalisation or resulted in hospitalisation

2. Wilson AP, Weavill C, Burridge J, Kelsey MC The use of the wound scoring method 'ASEPSIS' in postoperative wound surveillance. *J Hosp Infect* 1990;**16**:297-309.
3. Mehta RL, KellumJA, Shah SV, Molitoris BA, Ronco C, Warnock DG, Levin A. Acute Kidney Injury Network: Report of an Initiative to Improve Outcomes in Acute Kidney Injury. *Critical Care*. 2007; **11**:R31.
4. EuroQol Group. EuroQol - a new facility for the measurement of health-related quality of life. Health Policy 1990; 16: 119-208.

7 AMENDMENTS TO THE SAP

Previous version	Previous date	New version	New date	Brief summary of changes

SUPPLEMENTARY MATERIAL: ASEPSIS SCORES

In-hospital asepsis scores

For each wound used in the operation (a minimum of one – chest – and a maximum of six – chest, left leg, right leg, left arm, right arm, other, per patient) a wound specific in-hospital asepsis score is derived using the following steps:

1. A daily score is derived for each of the days that the wound was scored (ideally scored on three separate occasions), from the following:
   - If both filter questions (wound hot/wound wet) are "No" then the daily score is zero.
   - Otherwise the daily score is derived from summing the points awarded as follows for the four proportions of wound affects answers given on the CRF:

Proportion of wound affected:	**0%**	**<20%**	**20-39%**	**40-59%**	**60-79%**	**>80%**
Serous exudates	0	1	2	3	4	5
Erythema	0	1	2	3	4	5
Purulent exudates	0	2	4	6	8	10
Wound separation	0	2	4	6	8	10

   Note: *Any missing scores will be assumed to be 0, unless all four scores are missing and then the daily score will be set to be missing.*

2. Data collection is ideally performed on days 3, 5 and 8 post-operatively. The following rules are used to determine if daily scores are valid:
   - A two day window is allowed either side of the intended day, so for example the day 3 score can be done between day 1 and day 5[4].
   - Any assessments done outside of these windows, after the date of discharge, or in an invalid order (e.g. day 5 done before day 3) are invalid and not used.
   - A minimum of one daily score is required to proceed further. If this is not the case then the in-hospital asepsis score for that wound is missing.
3. Scores for days 1 to 10 are calculated; scores for missing days are either propagated from the nearest score or interpolated between scores. Note that the actual day of assessment is used rather than the intended day. See the following examples:

[4] Note the day 8 score is intended to be performed on day 8 or, if the patient discharged sooner, on the day of discharge. Therefore if the patient is discharged prior to day 8 the allowed window will be within two days of discharge (for example if the patient is discharged on day 6 the window will be day 4 to day 6)

Day (post-op)	EXAMPLE 1 Score	Rule	EXAMPLE 2 Score	Rule
1	3	Propagate	6	Propagate
2	3	Observed	6	Propagate
3	2.25	Interpolate	6	Observed
4	1.5	Interpolate	8	Interpolate
5	0.75	Interpolate	10	Observed
6	0	Observed	8	Interpolate
7	0	Propagate	6	Interpolate
8	0	Propagate	4	Interpolate
9	0	Propagate	2	Interpolate
10	0	Propagate	0	Observed

4. Any daily scores after day 7 are then discarded. The remaining scores are summed and then multiplied by 5/7 to give a single score representing five days' worth of daily asepsis scores.
5. The final in-hospital asepsis score for the wound is then calculated from adding points to the score derived from point 4 if any of the following events occurred at any time in the post-operative stay for that wound:
   - Antibiotics given for wound infection: 10 points
   - Isolation of bacteria: 10 points
   - Drainage of pus under local anaesthetic: 5 points
   - Drainage of pus under general anaesthetic: 10 points
   - Length of hospital day >14 days: 5 points

   Note: *any missing elements will be assumed to be 0.*

Post-discharge asepsis scores

Post-discharge asepsis scores are calculated by taking the in-hospital asepsis score for each wound and adding additional points if the patient has answered the questions on the 3-month follow-up questionnaire for that wound as follows:

- Been given antibiotics for wound infection=Yes AND patient did not have antibiotics for wound infection in initial hospital admission: 10 points
- Doctor opened/drained an abscess=Yes AND patient did not have drainage of pus under local anaesthetic in initial hospital admission: 5 points
- Wound been opened and cleaned under general anaesthetic in hospital=Yes AND patient did not have drainage of pus under general anaesthetic in initial hospital admission: 10 points

- Wound discharged pus=Yes AND the purulent exudates question on the in-hospital questionnaire was no/missing at all time points: 5 points
- District nurse had to dress wound=Yes AND patient did not have drainage of pus under local anaesthetic in initial hospital admission: 5 points

Note: *any missing elements will be assumed to be 0.*