Efficacy of propofol-supplemented cardioplegia on biomarkers of organ injury in patients having cardiac surgery using cardiopulmonary bypass: Propofol cardioplegia for myocardial protection randomised controlled trial: the PROMPT2 Study

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Glossary

AABGI	Association of Anaesthetists of Great Britain and Ireland
ACTACC	Association for Cardiothoracic Anaesthesia and Critical Care
AE	Adverse event
AKI	Acute kidney injury
AR	Adverse reaction
ARDS	Acute respiratory distress syndrome
BHF	British Heart Foundation
CA	Cardioplegic arrest
CABG	Coronary artery bypass grafting
CPB	Cardiopulmonary bypass
CRF	Case report form
CROQ	Coronary revascularisation outcome questionnaire
CTEU	Clinical Trials and Evaluation Unit
cTnT	Cardiac troponin T
DMSC	Data monitoring and safety committee
EQ-5D-5L	EQ-5D-5L quality of life questionnaire
GCP	Good Clinical Practice
ICU	Intensive care unit
LV	Left ventricular
MACE	Major cardiac adverse event
MHRA	Medicines and healthcare products regulatory agency
mPTP	Mitochondrial permeability transition pore

MI	Myocardial infarction
NaCl	Sodium chloride
NIHR	National Institute for Health Research
PAR	Population attributable risk
PIL	Patient information leaflet
QoL	Quality of Life
RCT	Randomised controlled trial
REC	Research ethics committee
ROS	Reactive oxygen species
SAE	Serious adverse event
SAR	Serious adverse reaction
SCTS	Society of the Cardiothoracic Surgeons of Great Britain and Ireland
SPCS	Society of Clinical Perfusion Scientists of Great Britain and Ireland
SIRS	Systemic inflammatory response syndrome
SmPC	Summary of product characteristics
SOP	Standard operating procedure
SSAR	Suspected serious adverse reaction
SUSAR	Suspected unexpected serious adverse reaction
TMG	Trial management group
TSC	Trial steering committee

1. Trial summary

Almost 2.3 million people in the UK are living with heart disease and >36,000 cardiac surgery operations are carried out each year. During surgery the heart is isolated from the rest of the circulation and a heart-lung machine is used to supply oxygen to the blood and pump it around the body. The heart is stopped and provided with nutrients by a liquid that is injected directly into the heart arteries. This liquid is called cardioplegia. This allows the surgeon to operate on the heart while it is still and not filled with blood but looking after the heart in this way during surgery is not ideal. The heart muscle can become short of oxygen, and when the heart is restarted, and blood starts to flow again the muscle can be harmed.

The damage is believed to be caused mainly by the formation of highly reactive molecules known as 'free radicals' in the heart muscle during the time it is short of oxygen. Propofol is a general anaesthetic widely used in cardiac surgery and research suggests that propofol could protect the heart muscle against damage from free radicals. We want to investigate whether adding propofol to the cardioplegic solution in patients having isolated coronary artery bypass grafting (CABG) surgery using the heart-lung machine is beneficial and if the benefit is greater the more propofol that is used.

We propose to conduct a study, known as a randomised controlled trial (RCT), in which patients undergoing CABG surgery are allocated by chance to a 'high' dose of propofol, a 'low' dose of propofol or no additional propofol. Every other aspect of care will stay the same. We will monitor all study patients to check that adding propofol to the cardioplegic solution is safe and an independent group of experts will review these data regularly. At the end of the study we will compare information about patients who received the different doses of propofol with information about patients who received no additional propofol to determine whether propofol is beneficial. We will do this by studying chemicals released by the heart and other organs in the body when they are damaged or stressed. We will measure these chemicals in blood samples taken before

and after the operation. We will also collect information on post-operative complications at 3 and 12 months and will collect data from patients on their quality of life (QoL) at 3 and 12 months.

If we find that adding propofol to the cardioplegic solution reduces organ damage, patients undergoing cardiac surgery may have a faster recovery and fewer major complications in the year following surgery. This should also lead to reduced costs for the NHS.

2. Background

2.1 Existing research

Myocardial protection with cardioplegic arrest is the most common method of myocardial protection during cardiac surgery with cardiopulmonary bypass (CPB). However, it causes global ischemia of the myocardium, making it susceptible to reperfusion injury [1, 2]. Metabolic and ionic homeostasis are disrupted by ischaemia [3]. Anaerobic metabolism leads to a build-up of lactic acid, which causes a rise in the concentration of intracellular sodium ions. Prolonged ischemia can also lead to calcium ion loading and ischaemia-induced sodium ion accumulation may contribute to osmotic-induced cell swelling, which can cause sarcolemmal damage [4-6]. Upon reperfusion, the renewed availability of oxygen leads to a surge in the formation of mitochondrial reactive oxygen species (ROS) and further calcium ion loading, both of which cause cardiomyocyte death by necrosis and apoptosis [7, 8]. In view of the suboptimal protection conferred by current cardioplegic techniques, alternative cardioplegia 'recipes' have been sought to reduce the generation of ROS and calcium ion loading or, ultimately, inhibit the opening of the mitochondrial permeability transition pore (mPTP) [7, 9].

Propofol is widely used during cardiac surgery [10]. In addition to its anaesthetic effect, studies in animal models have shown that direct coronary perfusion with propofol during the ischaemic period is cardioprotective and such protection is mediated by improving tissue antioxidant capacity and reducing lipid peroxidation [11, 12]. More importantly, propofol has been shown to protect against global normothermic ischaemia and during cold cardioplegic arrest, and that this protection was associated with less opening of mPTP [13]. Experiments in a clinically relevant pig model have also provided evidence supporting the use of propofol to protect the myocardium during cross-clamping [14], which prompted the design of our original PROMPT trial to investigate the efficacy of adding protocol to cardioplegia in reducing myocardial injury [15].

We believe the PROMPT trial in 101 patients is the first RCT to have evaluated propofol supplementation of the cardioplegia solution in patients undergoing coronary artery bypass graft (CABG) or aortic valve replacement (AVR) surgery. Adding propofol protected against ischemic reperfusion injury; there was, on average, a 15% lower cardiac troponin T (cTnT) release, equating to a difference of 60-90ng/l across the first 48 hours [16]. The effect was greater in the CABG patients compared to AVR (average 20% (n=61) vs 1% (n=40) reduction in cTnT), although the interaction between propofol supplementation and type of surgery was not statistically significant. This difference is small but worthy of further investigation. Having observed no harms attributable to propofol supplemented cardioplegia, the next step is to explore the efficacy of a higher dose. Additionally, intralipid supplementation, used as a placebo in PROMPT, may be cardioprotective [17-19], which would have reduced the apparent efficacy of the propofol supplementation. For this reason, we are proposing to use a sham placebo in PROMPT2 (see Section 5.6). Overall, 22% of patients in PROMPT experienced at least one serious adverse event (SAE), with similar numbers in the propofol and intralipid groups, which is consistent with other trials [20].

2.2 Risks and benefits

Potential benefits to patients include the possibility of less myocardial injury with the propofol supplementation, which would be expected to reduce risk of a major adverse cardiac event (MACE) up to 12 months after surgery [21]. Conversely, the patients randomised to the sham supplementation group may receive an inferior treatment (a possible harm of participating in any trial) though this would be the same for non-trial patients receiving standard care.

Patients will be randomised in a 1:1:1 ratio to sham supplementation, low dose supplementation with propofol or high dose supplementation with propofol, so they will all have an equal chance of being placed in each group and 67% chance of receiving propofol supplementation.

With respect to risk to patients from exposure to supplementary propofol above and beyond standard care:

- Propofol is widely used for induction and maintenance of general anaesthesia during cardiac surgery. It is an extremely safe anaesthetic agent with a very short half-life in the circulation.
- The levels of propofol supplementation in the cardioplegia solution [16] will be no higher than is already established as safe in the circulation when propofol is used to induce and maintain anaesthesia.
- The supplemented cardioplegia solution will be diluted approximately 15 to 20-fold when returned to the systemic circulation; the majority of supplementary propofol from one dose of cardioplegia will also have been cleared from the circulation by the time of the next dose (about 15-25 minutes later) [16]. Propofol clearance follows a three-compartment open model, with a first exponential phase half-life of approximately 1.6 to 4.0 minutes. This short half-life and rapid distribution results not only in fast onset of propofol action but short duration of action. The increased propofol dose given in the study will not lead to a significant accumulation in secondary compartments. We would expect the propofol half-life to continue to be less than 4.0 minutes.
- Any risk from supplementing the cardioplegia solution with propofol is anticipated to be small. Nonetheless, in order to ensure patients are not exposed to undue risk, blood samples will be taken at 6 hours and 24 hours after surgery to assess the safety/toxicity of propofol supplementation (see Section 5.9) for safety/toxicity outcomes and Section 7.2 for a description of the statistical analyses to be applied and the criteria for stopping recruitment on safety/toxicity grounds).

No additional pain, discomfort, distress or changes to lifestyle are expected for the patients allocated to any of the study groups. All patients will have the inconvenience of doing some extra tests e.g. completing health questionnaires before and after surgery. The potential benefits to society if propofol supplementation is found to be efficacious in reducing myocardial injury is that it may lead to faster recovery, reduced risk of MACE in the first post-operative year [21, 22] and reduced NHS costs.

3. Rationale

Almost 2.3 million people in the UK are living with some form of coronary heart disease with more than 308 000 men and 250 000 women living with heart failure [23]. Additionally, over 36,000 cardiac surgery procedures were carried in 2013, which included 17,630 first time isolated CABG procedures [24].

Complications after cardiac surgery are common. In a recent RCT conducted by our research team in 2000 patients undergoing cardiac surgery [20], 553/816 (68%) patients having CABG experienced one or more adverse event (mean 1.9 events) and 37% had at least one SAE. SAEs were (by definition) either fatal or extended the duration of the hospital stay; overall, those who experienced any adverse event stayed in hospital on average 7 days compared with an average length of stay of 6 days for patients who did not experience any adverse events. Patients who experienced at least one SAE stayed 9 days on average. SAEs, as well as having direct resource consequences for the NHS in the immediate post-operative period, can also result in long-term sequelae. Poor myocardial protection during heart surgery can result in loss of myocardium and subsequent heart failure which can occur not only during the early postoperative period but also late after discharge as a consequence of left ventricular remodelling.

Clinically, cTnT is used to diagnose myocardial infarction (MI). MI is rare after cardiac surgery (<0.5%) and the average cTnT concentrations observed in the PROMPT trial were below the threshold used to diagnose a MI. However, several studies have shown that the lower levels of cTnT release are predictive of outcome. Petaja and colleagues demonstrated that the odds of a MACE in the first 30 days after CABG surgery increases by 9% for every 1mcg/l rise in cTcT [22], emphasising that the risk of MACE increases steadily as post-operative cTnT concentration increases. Lurati Buse and colleagues have further demonstrated that a cTnT concentration >0.8mcg/l increases the hazard of MACE in the year following surgery by 2.13 times [21]. Thus, if the findings of the PROMPT trial can be replicated and if a higher concentration of propofol to the cardioplegia solution further reduces cardiac injury (i.e. further reduction in cTnT release) this would be expected to reduce the incidence of MACE in the first post-operative year. An association between raised cTnT and increased risk of mortality has also been observed [25-27].

The results from the PROMPT trial demonstrating proof-of-concept in lowering post-operative cTnT and the evidence from previous studies of the increased risk of MACE associated with raised cTnT release provide the rationale for our proposed study. We wish to investigate the safety and efficacy of propofol supplementation at a higher concentration than used in the PROMPT trial, comparing this high concentration to the level used in PROMPT and to sham supplementation.

As well as evaluating the safety and efficacy of a propofol supplementation against established biomarkers of cardiac and renal injury, PROMPT2 will investigate emerging biomarkers believed to characterise better the proximal mechanisms affected by oxidative stress [28-33]. As well as replicating emerging findings about these biomarkers, studying them in PROMPT2 will provide more information about their association with conventional markers such as cTnT, their potential as more responsive or specific markers and about opportunities to develop novel therapies to target the underlying mechanisms.

4. Aims and objectives

The PROMPT2 study will compare the efficacy and safety of different concentrations of propofol added to the cardioplegia solution during cardiac surgery versus sham supplementation. We hypothesise that propofol supplementation reduces myocardial injury in patients undergoing isolated CABG surgery with CPB. The trial will also investigate new cardiovascular biomarkers of the effects of oxidative stress in myocardium (caused by ischaemia and reperfusion during and after CPB), namely selected microRNAs and exosomes in blood and other tissues.

Specific objectives of the RCT are to estimate:

A. The difference between groups in the cTnT response following surgery (biomarker of cardiac injury) and whether high dose propofol supplementation reduces cTnT more than low dose propofol.

B. The difference between groups with respect to a range of other biomarkers (e.g. biomarkers of renal injury, inflammation, oxidative stress and metabolic stress).

C. The frequencies of serious adverse events, and serious adverse reactions in the three groups.

D. The difference between groups in QoL at 3 and 12 months after surgery.

Specific objectives of the mechanistic biomarker studies are to

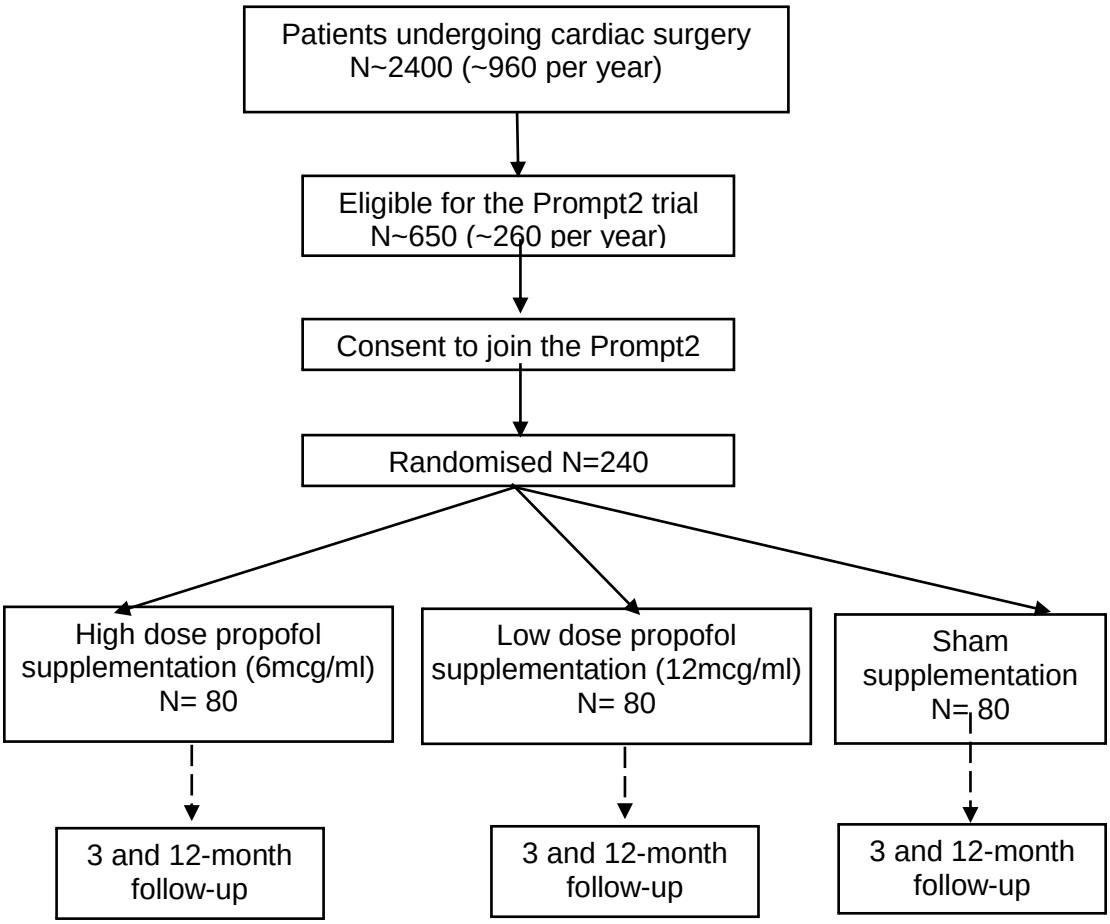
E. Investigate the association between cTnT and circulating level of cardiac-released microRNA-1 and exosomal microRNA-1 content.

F. Examine whether the association between cardiac troponin T and microRNA and exosomal microRNA-1 content differs between groups (i.e. differs with the propofol supplementation received)

5. Plan of Investigation

5.1 Trial schema

Figure 1 Trial schema



5.2 Trial design

This study is multi-centre study. The proposed evaluation will be a parallel three-group, sham-controlled RCT. Patients, clinical care teams, members of the research team responsible for data collection and laboratory personnel are blinded to allocation.

Phase 1: Set-up and recruit in two surgical centres with integrated monitoring and feedback to maximise recruitment and real-time monitoring for safety (see Section 5.9 for details).

Phase 2: Continue recruitment, using the optimum methods of recruitment established in Phase 1 for an additional 18 months following all participants (including those recruited during Phase 1) to 12 months.

Progression will be contingent on meeting the criteria defined in Section 5.9.

5.3 Setting

Participants will be recruited from cardiac surgery centres in two or more NHS hospitals.

5.4 Key design features to minimise bias

a) **Bias arising from the randomisation process (selection/allocation bias)** (systematic differences between baseline characteristics of the groups that are compared) will be prevented by concealed randomisation. The allocation will not be revealed until sufficient information to uniquely identify the patient and confirm eligibility has been entered into the allocation database.

b) **Bias due to deviations from intended interventions (performance bias)** (systematic differences between groups in the care that is provided, or in exposure to factors other than the interventions of interest) will be minimised by blinding all participants, clinical care team and members of the research team (apart from the study statistician who prepares the randomisation scheme) to participants' allocation. The cardioplegia solution will be prepared by the perfusionist, without disclosing the allocation to other theatre staff. The patient information leaflet (PIL) and the process of obtaining informed consent will describe the uncertainty about the effects of cardioplegia supplementation with propofol. Therefore, in the event of inadvertent unblinding of a patient, he or she should not have a strong expectation that any one method should lead to a more favourable result.

c) **Bias in measurement of the outcome (detection bias)** (systematic differences between groups in how outcomes are determined) will be minimised by blinding all individuals assessing outcomes and by using outcome measures that are defined as far as possible on the basis of objective criteria. Biochemical markers, including the primary outcome, will be analysed using standardised laboratory protocols by laboratory personnel blinded to the allocation.

d) **Bias due to missing outcome data (attrition bias)** (systematic differences between groups in withdrawals from a study) will be minimised by using established methods developed in the Clinical Trials and Evaluation Unit (CTEU) to maximise the quality and completeness of the data and minimise treatment cross-overs (e.g. regular monitoring of data, detailed querying of data inbuilt into the study database, offering alternative methods for participating in follow-up (e.g. postal, online or telephone)). Any instances of non-adherence will be fully documented and reviewed at study meetings and an action plan for maximising compliance drawn up as appropriate. Data will be analysed by intention to treat (i.e. according to the treatment allocation, irrespective of future management and events), and every effort will be made to include all randomised patients. The primary outcome is measured within 48 hours after the operation when participants are still inpatients so missing data for the primary outcome is not anticipated.

e) **Bias in selection of the reported result (reporting bias)** will be minimised by pre-specifying study outcomes and following a detailed analysis plan which will be prepared in advance of any comparative analyses of the study data.

5.5 Trial population

Cardioplegia supplemented with propofol can, in principle, be applied to virtually all adult patients having CABG with CPB. Therefore, adults undergoing elective or urgent CABG surgery for the first time at a specialist cardiac surgery centre represent the target study population.

5.5.1 Inclusion criteria

Patients may enter the study if ALL of the following apply:

1. Age ≥18 years
2. Having elective or urgent isolated CABG with CPB
3. Ability to give informed consent

Women only:

4. Negative pregnancy test, or be surgically sterile or post-menopausal for >12 months

5.5.2 Exclusion criteria

Patients may not enter the study if ANY of the following apply:

1. Previous cardiac surgery
2. Planned concomitant procedure
3. Emergency or salvage operation
4. Long-term steroid therapy (taking tablets on a daily basis for at least 1 month prior to surgery)
5. Pre-operative estimated glomerular filtration rate ≤ 60 mls/min/1.73m^2
6. Current congestive heart failure
7. Left ventricular (LV) ejection fraction <30% (i.e. poor LV function)
8. Allergy to peanuts, eggs, egg products, soybeans or soy products
9. Already participating in another interventional clinical study
10. Prisoners

Women only:

11. Breast feeding

5.6 Trial interventions

Study patients will be randomly allocated in a 1:1:1 ratio to one of the following:

Placebo: Blood cardioplegia with sham supplementation (normal saline 0.9% weight/volume sodium chloride, NaCl)

Propofol - LD: Blood cardioplegia with low dose (6mcg/ml) propofol supplementation

Propofol - HD: Blood cardioplegia with high dose (12mcg/ml) propofol supplementation

The lower dose (6mcg/ml) was used in the PROMPT trial [16] and showed some evidence of a cardioprotective effect. To investigate if there is a dose-response relationship a second higher dose (double the low dose concentration) is also being investigated. This higher dose was chosen to increase the concentration of propofol in the circulation and is within the range of plasma concentrations routinely attained during induction and maintenance of anaesthesia in surgery.

To maintain anaesthesia, most patients will require plasma concentrations of propofol around 2 – 6mcg/ml [40]. During anaesthesia for cardiac surgery, because of high-dose opiate use, the plasma concentration is towards the lower end of this range. There is no published maximum plasma concentration for maintenance of anaesthesia. However, standard target controlled infusion software, such as Diprifusor® (Smiths Medical, Kent. UK) has a maximum plasma target of 15mcg/ml.

5.6.1 Propofol composition and dispensing

Lipuro® 1% propofol emulsion for injection or infusion, B. Braun. 1ml emulsion contains 10mg propofol.

Excipients: Soya-bean oil, refined; triglycerides medium chain; purified egg phosphatides; glycerol; oleic acid; sodium hydroxide and water for injections.

Propofol for use in the study will be labelled and supplied by the local hospital pharmacy department in accordance with Good Clinical Practice (GCP). Propofol will be stored in a restricted access area that is temperature controlled and monitored. Drug accountability will be managed via a study-specific database. A risk assessment will be undertaken as per CTEU standard operating procedures. Further information about blinding/randomisation is detailed in section 6.1 and 6.2 of the protocol.

5.6.2 Anaesthetic regimen

To maintain consistent plasma concentrations of systemic propofol, anaesthetic management will adhere to the following protocol:

a) Induction: (i) 0 - 1.0mg/kg propofol, (ii) fentanyl OR alfentanil and (iii) a muscle relaxant;
b) Maintenance: (i) Isoflurane 0.5 - 2.0% in air/O2, (ii) plus additional opiate as required and (iii)Intravenous propofol infusion from the time of full heparinisation to maintain anaesthesia on bypass: 5 - 8mg/kg/hr
c) Post-operatively: (i) Propofol 2-7mg/kg/hr until extubation

All other aspects of the patient's pre- and post-operative management will be in accordance with existing clinical protocols.

5.6.3 Pharmacokinetics of propofol used for induction and maintenance of anaesthesia

Propofol is recommended for induction of general anaesthesia at a rate of 20-40 mg every 10 seconds with most patients requiring 1.5-2.5mg/kg. For an 80kg adult this equates to a total of 120-200mg and assuming a total blood volume of 6l this will lead to an initial plasma concentration of 20-33mcg/ml. In the context of cardiac anaesthesia, where larger doses of opiate are used, induction doses are significantly smaller (usually 0.25–1.0mg/kg). This would equate to a total induction dose of 20–100mg (initial plasma concentration 3.3–16mcg/ml). For maintenance, a rate of 4-12mg/kg/hr is recommended in the British National Formulary targeting a plasma concentration of 2.6–7.7mcg/ml. This assumes a linear relationship between infusion rate and plasma concentration that has been demonstrated based on measurements in clinical studies [41]. Propofol maintenance during CPB is common practice at the study centres and a rate of 33-100mcg/kg/min (equating to 1.98-6mg/kg/h) is commonly used. The current maintenance protocol would subsequently result in circulating plasma propofol concentrations of between 1.3-3.6mcg/ml.

5.6.4 Propofol supplementation of cardioplegia

Stock propofol (1%: 10,000mcg/ml) will be diluted 1 in 5 with 0.9% saline (as recommended by the manufacturers) to achieve a working solution of 2000mcg/ml.

The syringe driver will be set to 0.6ml/min (low dose) or 1.2ml/min (high dose) resulting in a 6mcg/ml or 12mcg/ml supplementation of the blood/cardioplegia mix during delivery respectively.

5.6.5 Pharmacokinetics of propofol given in cardioplegia

During surgery, around 300-400ml of the blood/cardioplegia mixture will return to the circulation every 15 to 25 minutes. Assuming the patient has a circulating volume of 6l, this volume of cardioplegia solution will be diluted approximately 15 to 20-fold in the systemic circulation. The resultant rise in the circulating propofol concentration after each dose of cardioplegia will be very small. This equates to a rise in systemic propofol concentration of:

Low dose: 1800mcg total (6mcg/ml x 300ml) diluted in 6000ml circulation = 0.3mcg/ml

High-dose: 3600mcg total (12mcg/ml x 300ml) diluted in 6000ml circulation = 0.6mcg/ml

The half-life for the first phase of propofol clearing the plasma compartment is short (1.6-4.0 minutes [42]). Approximately 4 to 6 half-lives (15 – 25 minutes) will elapse between each dose of cardioplegia. Therefore, the elevated propofol concentration in the systemic circulation before the next dose will be close to zero. Thus, the systemic accumulation of propofol due to the cardioplegia supplementation will be negligible and the intended concentration in the cardioplegia will be the true concentration in the cardioplegia. From a safety perspective; this also means that the systemic concentrations of propofol will not rise above concentrations routinely attained during induction and maintenance of anaesthesia in surgery.

5.6.6 Supplementation procedure

The intervention (propofol or saline) will be added by attaching an additional syringe pump to the line downstream of the blood oxygenator. This method is identical to that used for adding potassium (K^+) and magnesium (Mg^{2+}) to the oxygenated blood.

5.6.7 Standard cardioplegia composition and delivery

Blood cardioplegia will be used. This comprises Potassium Chloride (KCl) (15%) 2mmol K^+/ml and Magnesium Sulphate ($MgSO_4$) (50%) 2mmol Mg^{2+}/ml, mixed in a K^+:Mg^{2+} 4:1 ratio.

A 60ml syringe is prepared with 20ml KCl and 5ml $MgSO_4$ and is loaded into a syringe driver. A roller pump draws oxygenated blood from the oxygenator and the K^+/Mg^{2+} mixture is added by syringe pump downstream. Intermittent antegrade delivery will be used according to local protocol.

5.6.8 Sham supplementation

Normal Saline (0.9% weight/volume of NaCL) supplementation of the blood/cardioplegia delivered with the syringe driver will be set to 1.2ml/min (same as high dose propofol).

5.6.9 Treatment adherence

Withdrawals during treatment should not occur due to the nature and duration of the intervention; the duration of intervention in the trial is the duration of CPB. Problems with adherence (e.g. giving rise to cross-overs) are also expected to be low. In PROMPT, there were 7/101 protocol deviations; six patients did not receive any trial treatment, and there was one

cross-over [16]. We will use our experience from PROMPT to minimise non-adherence in PROMPT2.

5.7 Primary and secondary outcomes

5.7.1 Primary outcome

The primary outcome is myocardial injury, assessed by serial measurements of cTnT in serum from blood samples collected pre-operatively and during the first 48-hours post chest closure.

5.7.2 Secondary outcomes

Data will be collected to characterise the following secondary outcomes:

1. Systemic metabolic stress as measured by blood lactate
2. Renal function, as measured by creatinine in serum
3. Markers of Inflammation and oxidative stress as measured by tumour necrosis factor (TNF)-alpha, interleukin (IL)-10, IL-8, IL-6 and myeloperoxidase (MPO) in serum (Bristol cohort only)
4. Blood pH
5. Investigate the association between cTnT and circulating level of cardiac -released microRNA-1 and exosomal microRNA-1 content.
6. Examine whether the association between cTnT and microRNA and exosomal microRNA-1 content differs between groups (i.e. differs with the propofol supplementation received)
7. Length of intensive care unit (ICU) stay
8. Length of postoperative hospital stay
9. Clinical outcomes and serious adverse events, i.e. serious post-operative complications (e.g. MI, permanent stroke, acute kidney injury) and death from any cause.
10. QoL measured using the Coronary Revascularisation Outcome Questionnaire (CROQ) and the EQ-5D-5L questionnaire

5.8 Sample size calculation

The target sample size for the RCT is 240 patients, 80 per group. Eighty patients per group will provide 90% power at a 5% significance level (2-sided) to detect a difference of 0.25 standard deviations (SD) in cTnT between adjacent groups in an overall analysis of the three groups (i.e. relative means for the three groups of 0, 0.25 and 0.50 respectively), allowing for 7.5% dropout (see Section 6.12). Additionally, the study will have 90% power to detect a moderate difference of 0.5 SD (target difference in PROMPT) between any two groups at the 1.67% significance level (Bonferroni adjustment for three comparisons).

Treatment effects of this magnitude would be expected to reduce the risk of MACE in the first year after surgery and are consistent with our experience in previous research. Fifty patients per group was sufficient for the observed difference in the first PROMPT trial (about 0.35 SDs) to be considered statistically significant [16].

In calculating the sample size, we have assumed that the correlation between the pre and post intervention measures of cTnT is 0.2 and that the correlation between five post intervention measures is 0.7. These correlation estimates were informed by the PROMPT trial. A study with one pre-randomisation and 5 post randomisation measures of cTnT, assuming these

correlations, gives a gain in efficiency (relative efficiency 1.389) compared to a study with a single post-intervention measurement of cTnT, effectively reducing the standard deviation (SD) by 16% (i.e. replacing SD by 0.84 x SD).

In addition to being used to diagnose a MI, cTnT is an important predictor of long term MACE [21]. In PROMPT2, we do not expect propofol supplementation to result in a significant reduction in the incidence of early post-operative MI because the incidence of MI is very low (<0.5%). However, the target difference in cTnT that the study will be able to detect represents a decrease in cTnT that might reduce the incidence of MACE in the first post-operative year by 25%. This population attributable risk (PAR) is based on the results for the control group in the PROMPT trial and evidence that a plasma cTnT concentration >0.8 mcg/l increases the hazard of MACE by 2.13 times [21]. Based on the means and SDs observed in the PROMPT trial, 34% of the intralipid group (vs 18% of the propofol group) had cTnT concentrations >0.8 mcg/l. Under the usual assumptions for a PAR, and interpreting the hazard ratio as a relative risk of ~2, the PAR is estimated as 0.25.

5.9 Stopping rules

There are two conditions that might lead to stopping the trial early.

1. A failure to recruit sufficient patients to meet the target sample size within the proposed recruitment period and refusal of the funder to extend the duration of recruitment.
2. Sufficient data have accrued to suggest that the trial is unsafe for one or more groups of patients.

With respect to (1), we plan to recruit our target sample size of 240 randomised patients over 30 months. We propose to formally review progress against this target after 12 months of recruitment. At this time if the target recruitment rate is being met 96 patients should have recruited. Accepting recruitment typically starts slowly and increases over time as the trial gets established and that there is some variability from one month to the next (e.g. recruitment is typically lower over Christmas and in the summer holiday period than at other times of year) we will propose halting the trial if

a) fewer than 72 patients have been recruited within 12 months and

b) the trial team are unable to provide a plan that satisfies the funder to make up the shortfall of 24 patients (i.e. the target recruitment of 5 patients per month is not being exceeded and all eligible surgeons are participating).

The trial team will prepare a report for the Trial Steering Committee (TSC) to consider and make a recommendation to the funder, NIHR-EME.

With respect to (2), the Data Monitoring and Safety Committee (DMSC) will monitor the early outcomes, namely cardiac and renal injury in the first 24 hours after surgery using cumulative sum (CUSUM) charts [34, 35] (see Section 7.2 for definitions and further details). CUSUM charts are routinely used to monitor early outcomes in other areas of medicine [36-39].

6. Trial methods

6.1 Description of randomisation

Randomisation will be carried out as close to the planned operation as possible, after eligibility has been confirmed and written informed consent given. Randomisation will be performed by a

member of the research team not involved in data collection using a secure internet-based randomisation system ensuring allocation concealment. Randomisation will take place as soon as it is confirmed the surgery will go ahead, which is typically 30 minutes before the patient is taken to theatre. Patients will be allocated in a 1:1:1 ratio to either i) high dose propofol supplementation or ii) low dose propofol supplementation or iii) sham supplementation. The allocation will be computer-generated and stratified by centre. Baseline quality of life (QoL) will be collected from the patients prior to randomisation.

6.2 Blinding

Patients, their clinical care team (i.e. their surgeon, anaesthetist, and those responsible for their post-operative care), research nurse(s) responsible for patient follow-up, and laboratory personnel will not be informed of the allocation. The person responsible for carrying out the randomisation will inform the perfusionist of the allocation and will liaise with the perfusionist to set up and deliver the intervention. Blinding was successfully achieved in the PROMPT trial; the allocation details and materials required for the intervention (e.g. bag of intralipid or vial of propofol) were handed to the perfusionist in a sealed opaque envelope, and removed from the operating theatre at the end of the procedure. The required volume of emulsion was drawn-up in a syringe by the perfusionist and added to the cardioplegia solution.

In PROMPT2 the control 'intervention' will be sham supplementation using a vial of saline, not intralipid that was used in PROMPT. Saline is a clear liquid and propofol is opaque; therefore, the infusion pump and line will be masked by a screen to avoid unblinding. It will not be possible for the surgeon (or anyone else in the operating theatre) to detect whether the supplement is saline or propofol since the mixture is incorporated into and heavily diluted in the blood, which is the main component of the cardioplegia.

6.3 Code breaking

If unblinding is requested during surgery, this will be facilitated by the perfusionist who is unblinded to the allocation. Unblinding requests after the participant leaves theatre can be managed via the paper CRF, the database or the IMP management system. Any such request will be fully documented including who requested the unblinding and the reason for unblinding. Unblinding rates will be monitored throughout the trial by the study team and by the independent DMSC that will be established to oversee patient safety in the trial (see Section 8.3).

Patients will be made aware before entering the study that they will not be told which treatment they will receive.

6.4 Research procedures

Patients will be required to do, or undergo, the following tasks or investigations specifically for the research:

Whilst in hospital:

- Read a PIL about the study (could be sent to the patient's home before the patient attends the hospital for their surgery)
- Give written informed consent to participate if willing to do so
- Complete 2 health status questionnaires prior to surgery
- Donate small blood samples before, during and after surgery

After discharge from hospital:

- Complete 2 health status questionnaires 3 months and 12 months after surgery.

6.5 Duration of treatment period

The duration of intervention in the trial is the duration of CPB.

6.6 Definition of end of trial

This trial consists of two phases: an interventional phase (the duration of CPB) and a follow-up phase (two follow-up visits after surgery; one at 3 months and a further follow-up at 12 months). The end of the trial for an individual patient is defined as completion of the 12-month follow-up. The definition of the end of the trial is the date when all patients have completed the 12- month follow-up or have been lost to follow-up, the database has been locked and the final analysis completed, and all biological samples have been destroyed or transferred to another study or biobank.

6.7 Data and sample collection

Data collection will include the following elements:

a) A log of patients approached for the trial .

b) A log of patients assessed against the eligibility criteria and, if ineligible, reasons for ineligibility.

c) Record of consent and baseline information (e.g. history and response to health status questionnaires) collected prior to randomisation.

d) Clinical outcome data collected using purpose-designed case report forms (CRFs). These forms will be completed during the patient's post-operative stay until hospital discharge.

e) QoL data collected pre-operatively (after consent and before randomisation) and again at 3 and 12 months. Patients will be able to complete follow-up questionnaires by post, on line or by telephone, according to their preference. Patients who fail to complete questionnaires within 2 weeks of it becoming due will be contacted/sent a reminder.

Table 1 Schedule of data collection – clinical and patient reported outcomes

	Pre-op	Intra-op	Post-op (up to hospital discharge)	Follow up	
				3 months	12 months
QoL questionnaires	✓			✓	✓
Clinical outcomes			✓		
Operation details		✓			
Post op complications			✓		
SAE collection		✓	✓	✓	✓

Where possible, blood samples will be taken from in situ lines or at the same time as routine bloods. The timing of each sample is shown in Table 2. cTnT and creatinine will be analysed in the local hospital laboratory from blood samples and lactate and pH will be analysed using a blood gas machine that is used as part of standard clinical care. The results of these samples

come from a print out in theatre and in CICU. They are also available on the Philips database system, situated in CICU. The printout is placed in the patient notes and are regarded as source data. The results for the creatinine samples are on the ICE database and this is considered as source data. Blood for the other biomarkers will only be collected for patients recruited in Bristol. These samples will be processed by UH Bristol clinical laboratory and then transferred to a University of Bristol storage facility (-80^{o}C freezer to ensure sample integrity. CTEU Bristol standard operating procedure for sample labelling will be used to ensure traceability. These biomarkers will be analysed in the University of Bristol research laboratories. The results of these samples will be generated via the database and regarded as the source data. Where consent is given, any samples remaining at the end of the study will be retained for future research (either under a new research Ethics application or within a Biobank).

Table 2 Schedule of data collection – biomarker outcomes

Biomarker	Blood Sample	Pre-op	10 mins post cross clamp release	Post chest closure				
				1hr	6hr	12hr	24hr	48hr
cTnT	Serum	✓		✓	✓	✓	✓	✓
Lactate	Whole blood	✓	✓	✓	✓	✓	✓	✓
Creatinine	Serum	✓		✓	✓	✓	✓	✓
TNF-alpha*	Serum	✓		✓	✓	✓	✓	✓
IL-6*	Serum	✓		✓	✓	✓	✓	✓
IL-8*	Serum	✓		✓	✓	✓	✓	✓
IL10*	Serum	✓		✓	✓	✓	✓	✓
pH	Whole blood	✓		✓	✓	✓	✓	✓
MPO*	Serum	✓		✓	✓	✓	✓	✓
microRNA-1*	Serum	✓		✓	✓	✓	✓	✓
exosomal microRNA-1*	Serum	✓		✓	✓	✓	✓	✓

* Bristol cohort only

6.8 Source data

Clinical outcome data will be collected using purpose-designed case report forms (CRFs). These forms will be completed during the patient's post-operative stay until hospital discharge. The primary data source for these data will be the patient's hospital notes. The laboratory reports will be the primary data source for the results of the biomarker outcomes. The completed patient questionnaires will be the primary data source for these measures.

6.9 Planned recruitment rate

The 240 study patients will be recruited over 30 months from two centres. Potential patients will be identified prospectively from a list of all patients having cardiac surgery. Study centres have agreed that patients eligible for participation in PROMPT2 will be prioritised for this study.

Using data from the Bristol cardiac surgery database, which captures data on all surgical procedures done as a basis, we estimate that 260 patients undergoing CABG each year would eligible for PROMPT2 across the two study centres. In the PROMPT trial 6% of eligible patients

were not invited to take part for clinical or logistical reasons and similar rates have been observed in other cardiac surgery trials run in Bristol. However, with two centres and more surgeons participating, the 'approach' rate may be lower, so we have increased the anticipated 'non-approach' rate from 6% to 25%, reducing the eligible patient pool to approximately 190/year.

We are anticipating that 50% of eligible patients will consent to take part, which is lower than 66% consent rate achieved in PROMPT, but higher than the average 35% achieved across 5 other adult cardiac surgery trials run in Bristol (n=3055 eligible patients, 1114 consented) that required more patient involvement. This equates to a projected recruitment rate across the two centres of 8/month.

6.10 Discontinuation/withdrawal of patients

There are no specific criteria for withdrawal. However, a clinician may withdraw a patient at any time if they feel it is in the patient's best interests. Reasons for withdrawal will be captured in the study database and reported. Similarly, a patient may request to withdraw at any time without giving a reason. A request for consent to use data collected up to the point of withdrawal will be made.

6.11 Frequency and duration of follow up

Patients are followed up twice; 3 and 12 months after surgery. After discharge from hospital, data concerning health status will be collected by questionnaires.

6.12 Likely rate of loss to follow-up

Until discharge from hospital, the only losses to follow-up will be due to death or patient withdrawal; these losses are expected to be very few (<2%). We expect loss to follow-up at 3 and 12 months to be minimal (no more than 7.5%); in PROMPT all patients were followed to 3 months for safety and 100/101 patients completed the QoL questionnaires at 3 months, and the Titre-2 trial (which randomised over 2000 cardiac surgery patients across 16 centres), achieved >95% follow-up to 3 months [20].

6.13 Expenses

There will be no expenses incurred by the patient and therefore reimbursement is not applicable.

7. Statistical analyses

7.1 Primary and secondary outcomes

Analyses will be performed on the intention-to-treat basis and will be directed by a pre-specified statistical analysis plan. Analyses will use data from all patients randomised. Continuous data will be summarized as mean and SD or median and inter-quartile range (IQR) if distributions are skewed. Categorical data are summarized as number and percentage.

Primary and secondary outcomes will be compared using logistic (binary variables), Cox proportional hazards (time to event variables), or linear mixed model (continuous variables

measured at multiple time points) regression, with sham supplementation as the reference group. Mixed models allow all patients with data to be included in the analysis, i.e. partial missing data (assumed missing at random) is permitted. Interactions between treatment and time will be examined and if significant at the 10% level, results will be reported separately for each post-operative time point; otherwise overall treatment effects will be reported. Model validity will be checked using standard methods; if a model is a poor fit, transformations will be explored. Outcomes analysed on a logarithmic scale were transformed back to the original scale after analysis and results presented as geometric mean ratios (GMR). Analyses will be adjusted for baseline values.

For each outcome, a model with indicators for the two propofol groups (i.e. assuming no ordering to the groups) will be compared with a model which assumes an ordinal linear dose response relationship with increasing propofol supplementation (i.e. as hypothesised, with the difference between the low dose and placebo being the same as the difference between the high and low dose). If there is no statistically significant difference in model fit between the two models, the dose-response model will be chosen (and the 'unordered group' model will not be pursued further). If there is a statistically significant improvement in fit, the model which assumes no ordering to the groups will be chosen. An overall assessment of the effect of treatment across the three groups will be reported and differences between pairs of treatments (e.g. sham vs. low dose supplementation, sham vs. high dose supplementation and low vs. high dose supplementation) will be quantified. Findings will be reported as effect sizes with 95% confidence intervals.

The trial is not powered to detect differences in clinical outcomes and their frequencies will be tabulated descriptively in accordance with guidelines for reporting RCTs [43].

7.2 Monitoring safety/toxicity outcomes

Biomarkers of myocardial and renal injury will be used to assess safety/toxicity in the first 24 hours after surgery (see Section 5.7). In particular, an 'event' will be considered to have occurred if **either** of the following conditions are met.

a) cTnT concentration >1000 ng/l in serum from blood samples at 6 or 24-hours post chest closure
b) Creatinine concentration ≥ 2 x baseline (pre-operative) in serum from blood sample at 24-hours post chest closure.

A separate CUSUM chart will be created by the a member of the statistics team for each study group but the identity of each group will remain masked and will only be revealed at the request of the DMSC. The chart is updated as each patient is recruited, and their early outcome known. Cardiac and/or renal injury in the first 24 hours will constitute an "event" for safety monitoring purposes. The target event rate (i.e. the expected event rate under standard care) will be estimated from past data and recommended chart parameters (i.e. the thresholds to signal alert and alarm) will be determined by simulation. The optimal choice of threshold minimises the number of events occurring before a true safety concern is detected while at the same time minimising false positive signals. The DMSC will formally review these recommended parameter estimates and recommend adoption or revision before the start of the trial.

For monitoring, patients will be grouped according to the treatment received, rather than by the intended treatment. If a chart signals an alert this will be notified to the DMSC and teleconference arranged to discuss the findings.

7.3 Mechanistic biomarker studies

The analyses of the association between cTnT and cardiac-released microRNA-1 and exosomal microRNA-1 content will be exploratory; the correlations between biomarkers will be investigated. Comparisons of biomarkers between treatment groups will follow the analysis plan outlined in section 7.1.

7.4 Subgroup analyses

No subgroup analyses are planned.

7.5 Frequency of analyses

The primary analysis will take place when follow-up is complete for all recruited patients. The value of including an interim analysis (e.g. to examine the dose-response relationship part way through the trial) will be discussed with the DMSC. Safety data will be reported to the DMSC every 6 months, together with any additional analyses the committee request. If a CUSUM chart signals an alert this will be notified to the DMSC immediately (see Section 7.2).

7.6 Criteria for the termination of the trial

The trial may be terminated early on the instruction of the TSC or if the results of another study supersede the necessity for completion of this study

7.7 Economic issues

There will be no formal economic analysis.

7.8 Combining the results of PROMPT and PROMPT2

The results of the first PROMPT trial will be combined with the PROMPT2 results network meta-analysis due to the different placebos used in the two trials (intralipid in PROMPT and saline here). The analysis plan for the network analysis will be developed with advice from Prof Higgins at the University of Bristol.

8. Trial management

The trial will be managed by the Clinical Trials and Evaluation Unit Bristol (CTEU Bristol). The CTEU Bristol is an UK Clinical Research Collaboration registered Clinical Trials Unit. The CTEU Bristol will prepare all the trial documentation and data collection forms, specify the randomisation scheme, develop and maintain the study database, check data quality as the trial progresses, monitor recruitment and carry out trial analyses in collaboration with the clinical investigators.

8.1 Day-to-day management

The trial will be managed by the CTEU Bristol. The trial will be managed by a Trial Management Group (TMG), which will meet face-to-face or by teleconference approximately every 6 weeks or as required. The TMG will be chaired by a Chief Investigator (or delegate) and will include members of the named research team.

8.2 Monitoring of sites

8.2.1 Initiation visit

Before the study commences training session(s) will be organised by CTEU Bristol. These sessions will ensure that personnel involved fully understand the protocol, CRFs and the practical procedures for the study.

8.2.2 Site monitoring

CTEU Bristol will carry out central monitoring of compliance of centres with the principles of Good Clinical Practice (GCP), the protocol and data collection procedures. The study database will have extensive in-built validation and the TMG will review the completeness and consistency of the data throughout the trial. CTEU Bristol will not check CRFs against the data entered or against source data, unless there are good reasons to visit the site to complete a monitoring visit (e.g. the central monitoring highlights a problem). As this is a blinded study any misclassification errors should have minimal impact on the study results. Site monitoring will be carried out by University Hospitals Bristol NHS Foundation Trust (UHBristol) on behalf of the Sponsor.

8.2.3 Site closeout

At the end of the study CTEU Bristol standard operating procedure for study closeout and archiving will followed.

8.3 Trial Steering Committee and Data Monitoring and Safety Committee

8.3.1 Trial Steering Committee (TSC)

An independent TSC will be established to oversee the conduct of the study. It is anticipated that the TSC will comprise the lead investigators, an independent chair and at least two additional independent members, at least one of whom will be a patient/public representative. The TSC will develop terms of reference outlining their responsibilities and operational details. The TSC will meet before recruitment begins and regularly (at intervals to be agreed with the Committee) during the course of the study. The TSC will formally review recruitment after 12 months and make recommendations (see Section 5.9).

8.3.2 Data Monitoring and Safety Committee (DMSC)

An independent DMSC will be established to review safety data during the course of the study and will advise on actions should the CUSUM chart signal. The DMSC will develop a charter outlining their responsibilities and operational details. The DMSC will meet (jointly with the TSC) before the trial begins and they will meet regularly thereafter (at intervals to be agreed with the Committee). Design of the CUSUM charts and the stopping rules proposed for the trial will be discussed at the first DMSC meeting, and decisions documented in the DMSC Charter.

8.4 Patient and public involvement

We will include PPI representation on the TSC and will actively involve the PPI group for the duration of the study, including the dissemination of the results.

9. Safety reporting

9.1 Definitions

An adverse event (AE) is any undesirable event in a subject receiving treatment according to the protocol, including occurrences which are not necessarily caused by or related to administration of the research procedures.

An adverse reaction (AR) is any undesirable experience that has happened a subject while taking a drug that is suspected to be caused by the drug or drugs.

A serious adverse event (SAE) is any event which result in death, is life threatening, requires hospitalisation or prolongs hospitalisation, results in persistent or significant disability or incapacity.

A suspected serious adverse reaction (SSAR) is any serious adverse event that is suspected to be related to the drug or drugs being taken.

Suspected unexpected serious adverse reaction (SUSAR) is an untoward medical occurrence suspected to be related to the drug or drugs being taken that is not consistent with the applicable product information and is serious.

9.2 Overview

Serious and other adverse events will be recorded and reported in accordance with GCP guidelines and CTEU Bristol's Serious Adverse Events and Safety Reporting Standard Operating Procedure (SOP-GE-012) (see Figure 2).

In surgery, post-operative complications are not unexpected and are not infrequent, often causing an extension of the patient's hospital admission. These complications are classified as 'anticipated' (see Table 4) and will not require expedited reporting to the Sponsor, Medicines and Healthcare products Regulatory Authority (MHRA) or Research Ethics Committee (REC).

Expected events are those associated with propofol, and are listed in the Summary of Product Characteristics (SmPC) (see section 9.3). Events that are fatal or life threatening will be treated as unexpected events.

The investigator will notify all SAEs to the Sponsor (and MHRA and REC as required), with the exception of known complications of surgery (i.e. events listed as 'anticipated' events in Table 4) that are not judged to be causally related to the study drug. Events that require expedited reporting will be reported to the Sponsor within 24 hours of knowledge of the event by the investigator. The Sponsor delegates assessment of expectedness and relatedness to the investigator but will also review each reported SAE and confirm this assessment.

All AEs during the participant's hospital stay and SAEs after hospital discharge will be recorded in detail on a CRF. At the conclusion of the study, all AEs recorded during the study will be

subject to statistical analysis, and the analysis and subsequent conclusions will be included in the final study report.

For all SAEs that require expedited reporting and are ongoing at the time of the initial report, the subject will be actively followed up, and the investigator (or delegated person) will provide a follow-up report five working days after the initial report and further follow-up reports as new information becomes available until the SAE has resolved.

Further elective surgery or intervention(s) during the follow-up period that were planned prior to recruitment to the trial will not be reported as an SAE.

Safety/toxicity in the immediate post-operative period will be assessed from blood samples taken at 6 and 24 hours. The results of these biomarker analyses will be entered into the study database and the CUSUM chart for the monitoring of toxicity will be updated (see Section 7.2).

9.3 Expected adverse events associated with the study medication

The SmPC for Lipuro® 1% propofol emulsion for injection or infusion, B. Braun forms the reference safety information and is approved by the MHRA for this trial.

9.4 Anticipated events associated with surgery

The AEs 'anticipated' in patients undergoing cardiac surgery are listed in Table 3.

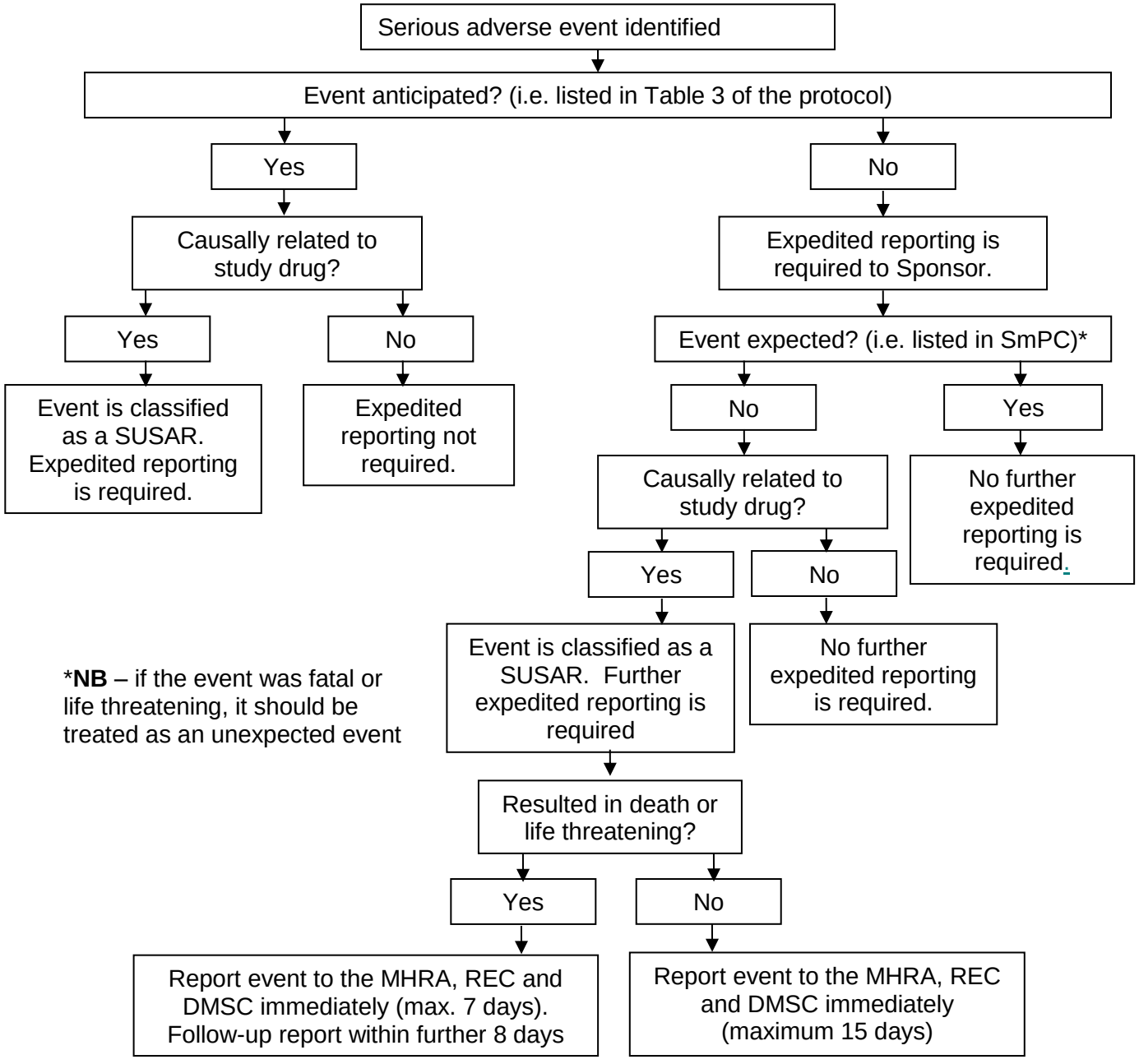
Table 3 Anticipated adverse events of surgery

Body system	Anticipated event
Cardiovascular	Myocardial Infarction Cardiac arrest, requiring: • Resuscitation involving ventricular defibrillation / direct current (DC) shock • Chest reopening • External/internal cardiac massage Haemodynamic support, including use of: • Any inotropes • Intra-aortic balloon pump (IABP) • Pulmonary artery catheter • Vasodilator Arrhythmias, including: • Supraventricular tachycardia / atrial fibrillation / atrial flutter • Ventricular tachycardia • Requirement for pacing • Requirment for Implantable cardioverter defibrillator (ICD) Chest pain Bleeding (needing re-operation or not) Deep vein thrombosis (DVT) Pulmonary embolus (PE) Pericardial effusion / tamponade Congestive heart failure requiring treatment

Body system	Anticipated event
	Pericarditis requiring treatment
	Blood clots
	Haematoma
	Peripheral thrombophlebitis
Pulmonary	Intubation / re-intubation and ventilation
	Tracheostomy
	Initiation of mask continuous positive airway pressure (CPAP) or non-invasive ventilation
	Acute respiratory distress syndrome (ARDS)
	Pneumothorax
	Pleural effusion
	Requirement for high flow oxygen
	Atelectasis / pulmonary collapse
	Surgical emphysema requiring intervention
	Bronchopleural fistula
	Prolonged air leak
	Chylothorax
	Acute aspiration
	Tracheobronchial injury
Renal / Urology	New haemofiltration / dialysis
	Urinary retention requiring reinsertion of urinary catheter, delaying discharge, or discharge with urinary catheter
Infective	Sepsis
	Wound infection
	Respiratory infection
	Medistinitis
	Urinary Tract Infection (UTI)
	Unspecifed infection
	Other infections requiring antibiotics
Gastrointestinal	Peptic ulcer / GI bleed / perforation
(GI)	Diagnostic laparotomy / laparoscopy
	Ischaemic bowel requiring treatment,
	Pancreatitis
	Delayed gastric emptying requiring intervention or delaying discharge or requiring maintenance of nasogastric drainage >7 days post-operatively
	Ascites
	New onset diabetes
Neurological /	Permanent stroke
Psychiatric	Transient ischaemic attack (TIA)
	Fitting/seizure
	Recurrent laryngeal nerve damage
	Other neurological injury
Re-operation	Re-operation due to any cause
	Wound dehiscence

Body system	Anticipated event
Anatomical / surgical damage	Incisional hernia
	Genital/renal tract injury
	Bronchoscopy for any cause
	Chyle leak / chylous ascites

Figure 2 Serious adverse event reporting flow chart



NB for any event where expedited reporting is required, this is the Sponsor in the first instance (within 24 hours of knowledge of the event) and to other parties as required.

9.5 Period for recording serious adverse events

Data on adverse events will be collected from the start of surgery for the duration of the patient's post-operative hospital stay and for the 12-month follow-up period.

10. Ethical considerations

All potential patients will be sent or given an invitation letter and PIL, approved by the HRA/NHS REC, describing the study. The PIL will outline the risks and benefits of taking part.

Typically, patients will have at least 24 hours to consider whether to participate. Occasionally, urgent in-patients waiting in one of the "referral" hospitals are transferred to the cardiac centre late and their surgery is due to take place within 24 hours of their arrival. We will make every effort to ensure such patients have adequate time to consider the trial by sending the study information to the feeder hospital for the patient to consider. Patients who have less than 24 hours to consider the study will only be consented if the patient feels that further deliberation will not change their decision, and provided the person seeking consent is satisfied that the patient has fully retained, understood and deliberated on the information given.

Suitably experienced and trained members of the local research team (e.g. study clinician/ research nurse/trial co-ordinator), all of whom are GCP trained, will be responsible for identifying eligible patients and obtaining written informed consent, as recorded on the Delegation Log. All potential patients will be seen by a member of the local research team who will explain the study, answer any questions, check the patient's eligibility and take written informed consent if the patient decides to participate. A copy of the signed Informed Consent Form will be given to the study patient. The original signed consent form will be retained at the study site, and a copy will be filed in the medical notes. Eligibility will be confirmed by a clinician prior to randomisation.

10.1 Risks and anticipated benefits

The risks and benefits of supplementing the cardioplegia solution with propofol are outlined in Section 2.2

10.2 Informing potential study patients of possible benefits and known risks

Information about possible benefits and risks of participation will be described in the PIL.

10.3 Obtaining informed consent from patients

All patients will be required to give written informed consent. This process, including the information about the trial given to patients in advance of recruitment, is described above

The research nurse/trial coordinator/study clinician will be responsible for the consent process.

10.4 Co-enrolment

Participants will not be permitted to co-enrol in the PROMPT2 study if they are receiving active drug therapy as part of the interventional phase of another clinical trial of an investigational medicinal product (CTIMP). Participants will not be permitted to co-enrol in other CTIMP trials during the interventional phase of the PROMPT2 trial. Participants will be permitted to take part

in other interventional or non-interventional studies (e.g. observational studies) as long as the burden placed on the patient is reasonable and the other trial protocol permits this (to be agreed on a trial by trial basis).

11. Research governance

This study will be conducted in accordance with:

- The Medicine for Human Use (Clinical Trial) Regulations 2004
- GCP guidelines
- UK Policy Framework for Health and Social Care Research
- European Union Directive 2001/20/EC on clinical trials

Annual reports will be submitted in line with regulatory requirements. Protocol breaches and deviations will be captured in the study case report forms and when required (e.g. serious breach) will be escalated to the Sponsor

11.1 Sponsor approval

The study protocol and patient documents including any amendments must be approved by the Sponsor, TSC (if requested) and funder prior to submission to the HRA/REC/MHRA.

11.2 NHS confirmation of capacity and capability

Confirmation of capacity and capability from each NHS Trust participating in the trial is required prior to the start of the trial at that site.

Any amendments to the trial documents approved the HRA, REC and/or MHRA will be submitted to the Trust confirmation of continued capacity and capability as required by the HRA classification of the amendment.

11.3 Investigators' responsibilities

Investigators will be required to ensure that local confirmation of capacity and capability has been obtained and that any contractual agreements required have been signed off by all parties before recruiting any patient. Investigators will be required to ensure compliance to the protocol and with completion of the CRFs. Investigators will be required to allow access to study documentation or source data on request for monitoring visits and audits performed by the Sponsor or CTEU Bristol or any regulatory authorities.

Investigators will be required to read, acknowledge and inform their trial team of any amendments that they receive and ensure that the changes are complied with.

11.4 Monitoring by Sponsor

The study will be monitored and audited in accordance with the Sponsor's policy, which is consistent with the UK Policy Framework for Health and Social Care Research and the Medicines for Human Use (Clinical Trials) Regulations 2004. All study related documents will be made available on request for monitoring and audit by the Sponsor, CTEU Bristol, the relevant REC and for inspection by the MHRA or other licensing bodies.

11.5 Indemnity

This is a University of Bristol-sponsored research study. If there is negligent harm during the clinical trial when the NHS body owes a duty of care to the person harmed, NHS Indemnity covers NHS staff, medical academic staff with honorary contracts, and those conducting the trial. NHS Indemnity does not offer no-fault compensation and is unable to agree in advance to pay compensation for non-negligent harm. Ex-gratia payments may be considered in the case of a claim.

11.6 Clinical Trial Authorisation

Propofol is classed as an investigational medicinal product and a Clinical Trial Authorisation from the MHRA must be in place before starting the trial.

12. Data protection and patient confidentiality

12.1 Data protection

Data will be collected and retained in accordance with the UK Data Protection Act 2018, the General Data Protection Regulation and associated UK law.

12.2 Data handling, storage and sharing

12.2.1 Data handling

Data will be entered into a purpose-designed server database hosted on the NHS network. Information capable of identifying individuals and the nature of treatment received will be held in the database with passwords restricted to PROMPT2 study staff at the participating site and the co-ordinating centre. Information capable of identifying participants will not be made available in any form to those outside the study.

Access to the database will be via a secure password-protected web-interface. Study data transferred electronically to the University of Bristol network for statistical analyses will be pseudonymised and transferred via a secure network. The participants will be identified using their name and unique study identifier on the secure NHS hosted database.

Data will be entered promptly and data validation and cleaning will be carried out throughout the trial. The trial manual will cover database use, data validation and data cleaning.

12.2.2 Data storage

All study documentation will be retained in a secure location during the conduct of the study and 5 years after the end of the study, when all patient identifiable paper records will be destroyed by confidential means. Where trial related information is documented in paper medical records, these records will be identified by a label bearing the name and duration of the trial. If paper records are no longer in use at a specific site, trial participation must be recorded using the appropriate local electronic system. In compliance with the MRC Policy on Data Sharing, relevant 'meta'-data about the trial and the full dataset, but without any participant identifiers other than the unique participant identifier, will be held indefinitely (University server).

12.2.3 Data sharing

Data will not be made available for sharing until after publication of the main results of the study. Thereafter, anonymised individual patient data will be made available for secondary research, conditional on assurance from the secondary researcher that the proposed use of the data is compliant with the MRC Policy on Data Sharing regarding scientific quality, ethical requirements and value for money. A minimum requirement with respect to scientific quality will be a publicly available pre-specified protocol describing the purpose, methods and analysis of the secondary research, e.g. a protocol for a Cochrane systematic review. The second file containing patient identifiers would be retained for record linkage or a similar purpose, subject to confirmation that the secondary research protocol has been approved by a UK REC or other similar, approved ethics review body. Patient identifiers would not be passed on to any third party. The data processor and controller is the University of Bristol.

13. Dissemination of findings

The findings will be disseminated by usual academic channels, i.e. presentation at international meetings, as well as by peer-reviewed publications and through patient organisations and newsletters to patients, where available.

We will link with and publicise the study through the Royal College of Anaesthetists, Royal College of Surgeons and the British Heart Foundation (BHF). There are annual meetings of the Society of the Cardiothoracic Surgeons of Great Britain and Ireland (SCTS), which most surgeons attend. An update on the study will be given at each meeting, to ensure that the surgical community is aware of the project and its progress. We will similarly publicise the study at annual meetings of the Association of Anaesthetists of Great Britain and Ireland (AAGBI), Association for Cardiothoracic Anaesthesia and Critical Care (ACTACC) and Society of Clinical Perfusion Scientists of Great Britain and Ireland (SCPS). Evidence from the first PROMPT trial and from PROMPT2 will be introduced as exemplars to students attending the MSc in Translational Cardiovascular Medicine and MSc in Perfusion Science (www. http://www.bristol.ac.uk/study/postgraduate/2017/health-sciences/msc-perfusion-science/), which has been established in collaboration with the SCPS, to further increase awareness of the research.

Additionally, social networking media will be used to disseminate and publicise the trial via a website and Twitter streams.

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15. Amendments to protocol

Amendment number (i.e. REC and/or MHRA amendment number)	Previous version	Previous date	New version	New date	Brief summary of change	Date of ethical approval (or NA if non-substantial)
NA	1.0	01/08/2018	2.0	24/09/2018	Updates to the eligibility criteria, unblinding information and safety section as per MHRA request	NA – part of the original approval process
1	2.0	24/09/2018	3.0		Simplification of anaesthetic protocol to reflect clinical practice Anticipated events added to Table 3 that were inadvertently missed	

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Disclaimer

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Appendix - SmPC

The current SmPC will be attached to the pdf version of this document