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REVIEW

Translating cardioprotection for patient benefit: position paper from the Working Group of Cellular Biology of the Heart of the European Society of Cardiology

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Abstract Coronary heart disease (CHD) is the leading cause of death and disability worldwide. Despite current therapy, the morbidity and mortality for patients with CHD remains significant. The most important manifestations of CHD arise from acute myocardial ischaemia–reperfusion injury (IRI) in terms of cardiomyocyte death and its long-term consequences. As such, new therapeutic interventions are required to protect the heart against the detrimental effects of acute IRI and improve clinical outcomes. Although a large number of cardioprotective therapies discovered in pre-clinical studies have been investigated in CHD patients, few have been translated into the clinical setting, and a significant number of these have failed to show any benefit in terms of reduced myocardial infarction and improved clinical outcomes. Because of this, there is currently no effective therapy for protecting the heart against the detrimental effects of acute IRI in patients with CHD. One major factor for this lack of success in translating cardioprotective therapies into the clinical setting can be attributed to problems with the clinical study design. Many of these clinical studies have not taken into consideration the important data provided from previously published pre-clinical and clinical studies. The overall aim of this ESC Working Group Cellular Biology of the Heart Position Paper is to provide recommendations for optimizing the design of clinical cardioprotection studies, which should hopefully result in new and effective therapeutic interventions for the future benefit of CHD patients.

Keywords Cardioprotection: Ischaemia • Reperfusion • Acute myocardial infarction • Cardiac surgery

1. Introduction

Coronary heart disease (CHD) is the leading cause of death and disability worldwide. According to the World Health Organisation (WHO), each year CHD accounts for the deaths of 3.8 million men and 3.4 million women. The global burden of CHD is projected to increase from 47 million DALYs (disability-adjusted life years or 'healthy years of life lost') in 1990 to ~82 million DALYs in 2020.

Many of the major complications of CHD, such as myocardial infarction (MI) and heart failure, arise from the detrimental effects of acute ischaemia–reperfusion injury (IRI) on the myocardium. As such novel therapeutic interventions are required to protect the myocardium against acute IRI in order to preserve cardiac contractile function, reduce the onset of heart failure, and improve clinical outcomes in patients with CHD. In this article, the term 'cardioprotection' is

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used to refer specifically to the protection of the myocardium against the detrimental effects of acute IRI. Over the years, the research field of cardioprotection has consistently failed to produce any effective therapeutic strategy for protecting the myocardium against acute IRI in the clinical setting. The failure has not been due to a shortage of potential cardioprotective strategies discovered in the pre-clinical experimental setting, but has been due to the inability to successfully translate many of these promising therapies into interventions that actually improve patient outcomes, a topic of much discussion in the recent literature.[1–4] In this regard, the overall aim of this ESC Working Group Cellular Biology of the Heart Position Paper will be to critically assess the translational process which takes place in the transition from the bench to the bedside, and to suggest recommendations for the future design of clinical cardioprotection studies, which take into consideration the important findings from both pre-clinical and clinical data in the research area of cardioprotection. Specifically, in this position paper we focus on the ways of optimizing the design of the clinical studies for testing novel cardioprotective interventions in two major clinical settings of acute myocardial IRI: patients presenting with an acute ST-segment elevation myocardial infarction (STEMI), treated by either thrombolytic therapy or primary percutaneous coronary intervention (PPCI) and patients undergoing coronary revascularization by coronary artery bypass graft (CABG) surgery. In particular, we critically analyse the contributions of patient selection, co-morbidities, concomitant medication, the timing of the therapeutic intervention, and the endpoints used for assessing cardioprotection, to the outcome of the clinical study. This should hopefully improve the chances of successfully translating future cardioprotective strategies for the benefit of CHD patients.

1.1 Major signalling pathways underlying cardioprotection

Elucidation of the major signal transduction pathways underlying endogenous cardioprotective strategies such as ischaemic preconditioning (IPC),[5] ischaemic postconditioning (IPost),[6,7] and remote ischaemic conditioning (RIC),[8,9] in which the heart is 'conditioned' either directly or indirectly by brief episodes of ischaemia and reperfusion, has identified two endogenous cardioprotective pathways, the Reperfusion Injury Salvage Kinase (RISK)[10,11] and the Survival Activating Factor Enhancement (SAFE) pathways.[12–15] These are recruited at the time of myocardial reperfusion and mediate cardioprotection. The RISK pathway includes the pro-survival kinase cascades MEK1/2-Erk1/2 and PI3K-Akt, whereas the SAFE pathway is made up by the TNF-α receptor and STAT3.[14–19] These two pathways relay the cardioprotective signal underlying the 'conditioning' strategies mentioned above, from cell membrane receptors to the mitochondria where protective mechanisms subsequently occur such as mitochondrial permeability transition pore (MPTP) inhibition,[20–23] mitochondrial connexin-43 channel activation, and mitochondrial ATP-dependent potassium channel opening.[24] The elucidation of these cardioprotective signalling pathways in pre-clinical studies has been pivotal in identifying therapeutic targets for cardioprotection in the clinical setting.

2. Opportunities for cardioprotection

In this section, the major clinical settings in the which the CHD patient is subjected to the detrimental effects of acute myocardial IRI and so potentially benefit from novel cardioprotective strategies, are reviewed.

2.1 Acute STEMI patients undergoing myocardial reperfusion

The clinical scenario, which most typically represents a classical example of acute myocardial IRI, is the patient presenting with an acute STEMI, treated by either thrombolytic therapy or PPCI.

In-hospital mortality of unselected STEMI patients in the national registries of the ESC countries varies between 6 and 14%.[25] There has been a reduction in both acute and long-term mortality following STEMI, due to greater use of reperfusion therapy, PPCI, anti-thrombotic therapy, and secondary prevention treatments, although the number of patients developing heart failure has increased.[26] However, despite this, mortality post-STEMI remains substantial with ~12% of patients being dead within 6 months,[27] with an increased mortality rate in higher-risk patients.[28] In developed countries, ~1–2% of the adult population suffer from heart failure, with the prevalence increasing to ≥10% among persons 70 years of age or older.[29] Therefore, these data underscore the importance of discovering novel therapeutic targets for protecting the heart against acute IRI so as to limit the MI size, prevent the onset of heart failure, and reduce cardiac mortality.

For patients presenting with an acute STEMI, early myocardial reperfusion using either thrombolytic therapy or PPCI remains the most effective treatment strategy for limiting the MI size, preserving cardiac function, and reducing the onset of heart failure. Where facilities are available, myocardial reperfusion by PPCI, as opposed to thrombolysis, is the preferred therapeutic strategy. Vast improvements have already been made in reducing the duration of acute myocardial ischaemia (the chest pain onset to PPCI time) with improved patient awareness (to reduce the time to first medical contact with the emergency medical services), minimizing the transit time to the PPCI centre, and reducing the door to PCI time at the PPCI centre.[30,31] Importantly, translation of such progress into improvement in patient outcomes has been documented.[32]

Improvements in both anti-platelet and anti-thrombotic therapy and advances in PCI technology to maintain the patency of the infarct-related coronary artery have further optimized the process of myocardial reperfusion. Although these therapeutic approaches clearly protect the coronary vasculature and reduce the risk of coronary re-thrombosis in PPCI patients, there is preliminary experimental evidence suggesting that both anti-platelet and anti-thrombotic therapy may actually confer direct protection on cardiomyocytes against acute IRI (see later section).

Paradoxically, the process of myocardial reperfusion can itself induce myocardial injury and cardiomyocyte death, a phenomenon which has been termed 'myocardial reperfusion injury'.[7,33] The reversible forms of myocardial reperfusion injury which include reperfusion arrhythmias and myocardial stunning are usually short-lived and easily managed.[7,33] However, the irreversible forms of myocardial reperfusion injury, which include microvascular obstruction (MVO) and lethal myocardial reperfusion injury ('reperfusion-induced necrosis'’),[34] contribute to the final myocardial infarct size and diminish the benefits of myocardial reperfusion in terms of myocardial salvage.[7,33] MVO describes the 'inability to reperfuse a previously ischemic region'.[35] The underlying cause of MVO is unclear although it has been attributed to capillary damage with

impaired vasodilatation, external capillary compression by endothelial cell and cardiomyocyte swelling, microembolization of friable material released from the atherosclerotic plaque, platelet microthrombi, and neutrophil adhesion and/or plugging.[36–40] Lethal myocardial reperfusion injury refers to the reperfusion-induced death of cardiomyocytes which were viable or reversibly injured at the end of ischaemia.[7,33,34] The mechanisms underlying this form of cardiomyocyte death are multiple and include oxidative stress, calcium overload, MPTP opening, cardiomyocyte hypercontracture, apoptosis, necrosis, necroptosis, and inflammation (reviewed in[7,33,41]).

2.2 Patients undergoing cardiopulmonary bypass surgery

Patients undergoing coronary revascularization by CABG surgery are subjected to global acute myocardial IRI. When the aorta is clamped prior to going onto cardiopulmonary bypass, the heart is made acutely ischaemic and when the heart is taken off cardiopulmonary bypass and the aorta is unclamped, the heart is subjected to acute myocardial reperfusion injury. This global acute myocardial IRI contributes to the peri-operative myocardial injury and infarction that occurs during CABG surgery. The incidence and magnitude of peri-operative myocardial injury and infarction can be measured using serum cardiac enzymes such as CK-MB,[42] Troponin-T,[43] and Troponin-I[44] and have been linked to worse clinical outcomes post-surgery. Guidelines for defining MI related to CABG have been recently published in the 'Third universal definition of myocardial infarction'.[45] Myocardial infarction related to CABG has been termed as Type 5 MI and has been defined as an elevation of cardiac biomarker values $>10 \times$ 99th percentile URL in patients with normal baseline cardiac Troponin values ($<$99th percentile URL), along with either (i) new pathological Q-waves or new left bundle branch block (LBBB), or (ii) angiographic documented new graft or new native coronary artery occlusion, or (iii) imaging evidence of new loss of viable myocardium or new regional wall motion abnormalities.[45]

Other factors that can result in peri-operative myocardial injury during CABG surgery include coronary embolization, manual handling of the heart, and inflammation.[46,47] As such, the discovery of novel cardioprotective strategies for minimizing this form of myocardial injury and infarction during CABG surgery would be expected to preserve cardiac function and improve clinical outcomes in this clinical setting, particularly in those high-risk patients who are most vulnerable to this form of myocardial injury and infarction.[48] Any cardioprotective intervention shown to be effective in the setting of CABG surgery may also be expected to be beneficial in other surgical settings in which the heart is subjected to acute global myocardial IRI, such as in major vascular and intra-abdominal surgery. In these latter settings, in which the pathophysiology of acute IRI is often unclear (and which include low cardiac output, coronary spasm, regional hypoperfusion, and so forth), additional studies are required to determine the relative contributions of acute ischaemia and reperfusion to the damage which occurs during surgery, in order to optimize cardiac protection.

2.3 Other opportunities for cardioprotection

2.3.1 Cardiopulmonary resuscitation

In a cardiopulmonary arrest, the whole body including the heart is subjected to acute global ischaemic injury. Successful cardiopulmonary resuscitation (CPR) results in the restoration of spontaneous circulation (ROSC) following the cardiac arrest which then subjects the whole body and the heart to acute global reperfusion injury. Following ROSC, the acute global myocardial IRI results in myocardial necrosis and post-resuscitation myocardial dysfunction, factors which, together with brain, kidney, and liver damage, are associated with worse clinical outcomes post-arrest.

There is an opportunity to administer a therapeutic intervention after the onset of cardiopulmonary arrest to minimize the acute global ischaemic injury and protect the heart and other vital organs. In this regard, a number of pre-clinical studies using animal models of cardiac arrest have investigated the role of a variety of cardioprotective interventions administered prior to cardiac arrest including mechanical interventions (therapeutic hypothermia[49]) and pharmacological ones [β-adrenergic blockade,[50] iNOS inhibition,[51] K_{ATP} channel activation,[52] sodium-hydrogen ion exchanger inhibitor,[53] erythropoietin,[54] and cyclosporin-A (CsA)[55]].

Importantly, a therapeutic intervention applied to protect the heart against acute IRI could also provide systemic organ-wide protection against acute IRI, benefiting the post-cardiac arrest function of other vital organs such as the brain, kidney, and liver. Clinical studies investigating novel cardioprotective strategies in the clinical setting of CPR are yet to be undertaken.

2.3.2 Cardiac transplantation

Acute myocardial IRI sustained during cardiac transplantation is a major cause of graft failure. In the setting of cardiac transplantation, the donor heart is subjected to cold myocardial ischaemic injury at the time of graft procurement, storage, and transportation, which exacerbates the inflammatory response and the chance of rejection, contributing to graft vasculopathy and failure.[56] At the time of graft implantation, injury to the graft is exacerbated by the acute global myocardial reperfusion injury which occurs on reperfusion of the graft.

There is an opportunity to administer a therapeutic intervention at the time of graft procurement, storage, and transport to minimize the cold ischaemic injury and protect the donor heart. Similarly, there is an opportunity to administer a therapeutic intervention to the recipient to protect the donor heart against acute global myocardial reperfusion injury that occurs at the time of graft implantation. In this regard, a number of pre-clinical studies have been published investigating a variety of cardioprotective interventions applied to the donor heart including pharmacological agents (adenosine analogue, sodium–hydrogen exchange inhibition, K_{ATP} channel activation, sildenafil, PKC-δ inhibition, and isoflurane) and mechanical interventions (IPC, IPost, and RIC) (reviewed in[56]). So far, no clinical studies have investigated cardioprotection in the setting of cardiac transplantation.

3. Optimizing the design of clinical cardioprotection studies

The failure to translate novel cardioprotective strategies discovered in pre-clinical studies into the clinical setting for patient benefit can be attributed to a number of different factors, the majority of which fall into three main categories: (i) the failure to develop a study intervention for human use against validated targets; (ii) inadequate or insufficient pre-clinical testing of the therapeutic intervention before

clinical testing; and (iii) the design of the clinical cardioprotection study.[1,3,4,57,58]

3.1 The study intervention

The first point to consider in planning a clinical trial on cardioprotection is the selection of the therapeutic intervention to be tested. Only treatments providing consistent and robust benefit in pre-clinical studies involving different models and laboratories should be considered. Although this may seem an obvious pre-requisite, the failure to take this factor into consideration has led to a large number of negative clinical trials (see *Table 1* for summary). This issue was discussed in a recent NHLBI Workshop and resulted in the formation of the CAESAR: NIH Cardioprotection Consortium, a network of research laboratories which are using a variety of clinically relevant pre-clinical animal MI models to test the efficacy of novel therapeutic agents to ensure they confer consistent and robust cardioprotection before entering the clinical arena.[3,59]

On the other hand, the translation to patients of pharmacological treatments for which there is strong pre-clinical evidence is often limited by the non-availability of drugs which can be used safely in humans, or the lack of interest in myocardial reperfusion injury by the companies who own these drugs. For example, pharmacological approaches which have solid pre-clinical evidence, but which lack drugs for human use are contractile blockers,[60] calpain inhibitors,[61] or particulate guanylate cyclase stimulators.[62] Even treatments which are available for human use have often been developed for other actions, many of which are undesired when applied to reduce reperfusion injury—an example of this is CsA, which was developed as an immunosuppressant agent and has been used to prevent myocardial reperfusion injury based on its effect on the MPTP (see *Table 2*). Overcoming these limitations will require a change in the perception of the pharmaceutical industry regarding the economic potential of developing and testing treatments against myocardial reperfusion injury.

Several of the failed study interventions listed in *Table 1*, including anti-oxidants, calcium-channel antagonists, adenosine, and erythropoietin had not shown conclusive cardioprotection in the pre-clinical animal studies, which may in part explain why they failed in the clinical setting. Another reason for the negative studies may be that many of them were designed to target only one proponent of myocardial reperfusion injury such as oxidative stress, calcium channel accumulation, apoptosis, and inflammation (see *Table 1*).

3.2 Experimental animal MI models

Many of the experimental animal MI models used to investigate study interventions in the pre-clinical setting do not adequately represent the clinical setting of a patient presenting with an acute MI undergoing myocardial reperfusion (for a summary of the major factors, see Supplementary material online, *Table S1*). This topic has been discussed in detail in several comprehensive reviews.[1,3,4,57,58]

3.3 Design of the clinical cardioprotection study

It is essential that the design of the clinical cardioprotection study takes into consideration the findings of previously published pre-clinical and clinical studies.

4. Confounding factors in STEMI cardioprotection studies

There currently exists no recognized effective therapeutic intervention for protecting the cardiomyocyte from the detrimental effects of either MVO or lethal myocardial reperfusion injury in acute MI patients. Over the last two to three decades, a large number of therapeutic interventions have been investigated as adjuncts to myocardial reperfusion. However, the results from the majority of these studies have been largely disappointing in terms of finding an effective therapy for reducing myocardial reperfusion injury and improving clinical outcomes in STEMI patients undergoing PPCI. *Table 1* provides a summary of the major clinical studies which have failed to demonstrate any benefit in reperfused STEMI patients, and highlights some of the potential reasons for their failure, many of which include not taking into account confounding factors to cardioprotection.

A number of novel therapeutic interventions have been reported in small proof-of-concept clinical studies to prevent lethal myocardial reperfusion injury in STEMI patients undergoing PPCI (*Table 2*). These include mechanical therapeutic strategies such as therapeutic hypothermia,[63] therapeutic hyperoxaemia,[64] IPost,[65] RIC,[66] and pharmacological therapies such as atrial natriuretic peptide (ANP),[67] CsA,[68] and exenatide.[69] Large multicentre clinical studies are now required to determine whether these promising therapeutic interventions can actually improve major clinical endpoints in STEMI patients treated by PPCI. In this regard, for CsA, RIC, and IPost these studies are currently underway (see Supplementary material online, *Table S2*).[70,71]

In addition to applying the cardioprotective strategy at the time of PPCI to prevent lethal myocardial reperfusion injury, there is also the opportunity of intervening at an earlier time-point, in the ambulance while in transit to the PPCI centre, in order to protect against acute myocardial ischaemic injury. This approach has recently been shown to be beneficial in proof-of-concept clinical studies investigating RIC and glucose–insulin–potassium therapy administered in the ambulance[66,72] and is currently being investigated using metoprolol (Ibanez *et al.* METOCARD-CNIC NCT01311700). *Table 3* provides a summary of some of the major therapeutic interventions which are currently being investigated as cardioprotective therapies for reducing lethal myocardial reperfusion injury in PPCI patients.

Based on extensive experimental data, and the findings from recent proof-of-concept clinical studies, particularly those which have investigated IPost in STEMI patients, our new understanding of the pathophysiology of acute IRI now allows us to propose recommendations for optimizing the design of clinical 'cardioprotection' trials. To increase our capacity to successfully transfer basic science knowledge into clinical practice for the patient's benefit, one may consider two distinct categories of confounding factors: (i) those factors which can be controlled for, and (ii) those that cannot be controlled for (see *Figure 1*). It is also important to realize that the confounding factors will vary according to the clinical situation, i.e. they are not the same for the STEMI and CABG setting.

4.1 Confounding factors which can be controlled for

Some factors are known as major determinants of MI size and must therefore be measured or taken into account in MI size reduction studies. Not doing so will either decrease the statistical power of the trial and/or result in a misinterpretation of the results, most

Table 1 Clinical studies which have failed to demonstrate any beneficial effect in STEMI patients with a therapeutic intervention administered at myocardial reperfusion

Clinical study	Therapeutic intervention	*n*, number	Outcome	Notes
	Anti-oxidant therapy			
EMIP-FR 2000[106]	IV bolus of trimetazidine given *prior* to thrombolysis followed by 48 h infusion	19 725	No difference in mortality at 35 days	Anterior STEMI only: no Only PPCI or thrombolysis: yes AAR measured: no Collateral flow excluded: no TIMI flow grade <1: no Treatment prior to or at reperfusion: yes
	Magnesium			
MAGIC[107]	IV bolus of magnesium given *prior* to reperfusion followed by 24 h infusion	6213	No difference in mortality at 30 days	Anterior STEMI only: no Only PPCI or thrombolysis: yes AAR measured: no Collateral flow excluded: no TIMI flow grade <1: no Treatment prior to or at reperfusion: yes
	Glucose insulin potassium (GIK) therapy			
Mehta *et al.*, CREATE-ECLA[108]	IV GIK infusion for 24 h started *after* reperfusion in the majority of cases	20 201	No difference in mortality at 30 days	Anterior STEMI only: no Only PPCI or thrombolysis: no AAR measured: no Collateral flow excluded: no TIMI flow grade <1: no Treatment prior to or at reperfusion: yes
	Sodium–hydrogen ion exchange inhibitors			
Zeymer *et al.*, ESCAMI[109]	Iv eniporide as a 10 min infusion *prior* to PPCI or *after* thrombolysis	2118	No difference in the MI size (72 h AUC alph-ahydroxybutyrate dehydrogenase)	Anterior STEMI only: no Only PPCI or thrombolysis: no AAR measured: no Collateral flow excluded: no TIMI flow grade <1: no Treatment prior to or at reperfusion: yes
	Nicorandil			
Kitakaze *et al.*, J-WIND-KTP[67]	Iv nicorandil bolus then 72 h infusion started *after* reperfusion	545	No difference in the MI size (72 h AUC total CK) or 6 month LVEF	Anterior STEMI only: no Only PPCI or thrombolysis: yes AAR measured: no Collateral flow excluded: no TIMI flow grade <1: no Treatment prior to or at reperfusion: no
	Anti-inflammatory agents			
Armstrong *et al.*, APEX-MI[110]	Iv pexelizumab bolus given *prior* to PPCI followed by infusion for 24 h	5745	No difference in all-cause death at 30 days	Anterior STEMI only: no Only PPCI or thrombolysis: yes AAR measured: no Collateral flow excluded: no TIMI flow grade <1: no Treatment prior to or at reperfusion: yes
Atar *et al.*, FIRE[111]	Iv FX06 bolus given *prior* to PPCI and then repeated 10 min later	232	No difference in the MI size by CMR at 5 days or 4 months	Anterior STEMI only: no Only PPCI or thrombolysis: yes AAR measured: no Collateral flow excluded: no TIMI flow grade <1: no Treatment prior to or at reperfusion: yes

Continued

Table 1 *Continued*

Clinical study	Therapeutic intervention	*n*, number	Outcome	Notes
	PKC-δ inhibitor			
Lincoff *et al.* 2011 PROTECTION-AMI, Unpublished	Iv delcasertib infusion for 24 h started *prior* to PPCI	1083		Anterior STEMI only: yes Only PPCI or thrombolysis: yes AAR measured: no Collateral flow excluded: yes TIMI flow grade <1: no Treatment prior to or at reperfusion: yes
	Erythropoietin (EPO)			Large animal studies inconclusive Potential off-target effects
Voors *et al.*, HEBE-III[112]	IV EPO epoetin-alpha 60 000 IU *after* (within 3 h) PPCI	529	No difference in the LVEF at 6 weeks. No difference in the MI size (AUC CK-MB or TnT) More major adverse cardiac events occurred with EPO	Anterior STEMI only: no Only PPCI or thrombolysis: yes AAR measured: no Collateral flow excluded: no TIMI flow grade <1: no Treatment prior to or at reperfusion: no
Ott *et al.*, REVIVAL-3[113]	IV EPO epoetin-beta 33 000 iU immediately *after* PPCI repeated 24 and 48 h later	138	No difference in LVEF at 6 months assessed by CMR. No difference in the MI size (5 days and 6 month CMR)	Anterior STEMI only: no Only PPCI or thrombolysis: yes AAR measured: no Collateral flow excluded: no TIMI flow grade <1: no Treatment prior to or at reperfusion: no
Ludman *et al.*[114]	IV EPO epoetin-beta 50 000 iU *prior* to PPCI repeated 24 h later	52	No difference in the MI size at 3 days using CMR and or 24 h AUC Trop T. Doubling of incidence of MVO on CMR	Anterior STEMI only: no Only PPCI or thrombolysis: yes AAR measured: yes Collateral flow excluded: no TIMI flow grade <1: no Treatment prior to or at reperfusion: yes
Rao *et al.* 2011, REVEAL NCT00378352	IV EPO epoetin-beta 60 000 iU immediately *after* PPCI repeated 24 and 48 h later	138	No difference in the MI size on CMR within 6 days and at 3 months	Anterior STEMI only: no Only PPCI or thrombolysis: yes AAR measured: no Collateral flow excluded: no TIMI flow grade <1: no Treatment prior to or at reperfusion: no
	Atorvastatin*			
Hahn *et al.*[115]	Oral atorvastatin 80 mg *prior* to PPCI and 10 mg daily thereafter	173	No difference in the MI size at 5–14 days using SPECT	Anterior STEMI only: no Only PPCI or thrombolysis: yes AAR measured: no Collateral flow excluded: no TIMI flow grade <1: no Treatment prior to or at reperfusion: yes
Post *et al.*, REPARATOR[116]	Oral atorvastatin 80 mg *prior* to PPCI and daily thereafter	42	No difference in LVESI at 30 days	Anterior STEMI only: no Only PPCI or thrombolysis: yes AAR measured: no Collateral flow excluded: no TIMI flow grade <1: no Treatment prior to or at reperfusion: yes
	Iron chelation			
Chan *et al.*[117]	Iv bolus of desferoxamine given *prior* to PPCI followed by 12 h infusion	60	No difference in the MI size (48 h AUC CK-MB and Trop I and CMR). No difference in myocardial salvage	Anterior STEMI only: no Only PPCI or thrombolysis: yes AAR measured: no Collateral flow excluded: no TIMI flow grade <1: no Treatment prior to or at reperfusion: yes

Continued

Table 1 *Continued*

Clinical study	Therapeutic intervention	*n*, number	Outcome	Notes
	Ischaemic postconditioning			
Tarantini *et al.*[100]	Four-60 s angioplasty balloon inflations/deflations	78	Non-significant increase in the MI size IPost protocol was delivered within the stent, increasing the risk of coronary microembolization	Anterior STEMI only: no Only PPCI or thrombolysis: yes AAR measured: no Collateral flow excluded: yes TIMI flow grade <1: yes Treatment prior to or at reperfusion: yes
Freixa *et al.*[101]	Four-60 s angioplasty balloon inflations/deflations	79	Reduced myocardial salvage. No difference in the MI size at 1 week or 6 months by CMR. IPost protocol delivered within the stent, increasing the risk of coronary microembolization	Anterior STEMI only: no Only PPCI or thrombolysis: yes AAR measured: no Collateral flow excluded: yes TIMI flow grade <1: yes Treatment prior to or at reperfusion: yes

Anterior STEMI only, only anterior STEMI patients included; only PPCI or thrombolysis, only either PPCI or thrombolysis patients included; AAR measured, area at risk measured; collateral flow excluded, coronary collateralization to the AAR excluded; TIMI flow grade <1, TIMI flow grade <1 in the infarct-related artery prior to PCI; treatment prior to or at reperfusion, study intervention given prior to or at reperfusion,*although oral atorvastatin was given prior to reperfusion, therapeutic levels would not have been achieved by this time.

often by concluding that the study is negative, thereby missing the opportunity for discovering new therapies for acute MI patients.

4.1.1 Patient selection

It must be appreciated that many of the clinical cardioprotection STEMI studies often exclude the most ill STEMI patients—these include those with critical life-threatening conditions such as cardiac arrest, cardiogenic shock, severe ventricular arrhythmias, and co-morbidities. In this regard, mechanical cardioprotective strategies, such as RIC, may be particularly beneficial in this patient group, as they have the potential to mediate multiorgan protection.

4.1.2 Choice of reperfusion strategy

One can hypothesize that the choice of reperfusion strategy between PPCI and thrombolysis may impact on the severity of MVO and lethal myocardial reperfusion injury experienced by the STEMI patients, and therefore have an effect on the cardioprotective efficacy of the study intervention. Pre-clinical data suggest that gradual or low-pressure reperfusion can limit the MI size when compared with unimpeded myocardial reperfusion.[73–75] In fact, this phenomenon[76] underlies the therapeutic basis of IPost, in which myocardial reperfusion occurs in a stuttered manner as it is interrupted by short-lived episodes of myocardial ischaemia, which has been reported to improve myocardial reperfusion, prevent endothelial dysfunction, reduce inflammation, attenuate apoptotic cell death, and limit MI size.[6] Therefore, in PPCI, where myocardial reperfusion occurs both abruptly and completely, one may expect there to be a greater degree of myocardial reperfusion injury when compared with thrombolysis, in which myocardial reperfusion takes place more gradually and less completely. Furthermore, the precise time and adequacy of reperfusion are unknown in patients treated with thrombolytic agents, uncertainties which will make it difficult to have comparable control and treatment groups. Alternatively, one should, however, acknowledge that previous trials directly comparing the efficacy of thrombolysis vs. PPCI in STEMI patients have not established that any form of IRI (e.g. MI size, clinical outcome) was significantly attenuated by thrombolysis with respect to PPCI. Yet, one cannot rule out that study interventions administered at the time of myocardial reperfusion may result in different outcomes depending on whether PPCI or thrombolytic therapy is employed to restore the coronary flow in the infarct-related artery. Therefore, clinical cardioprotection studies of STEMI patients should include only one of these two modes of reperfusion therapy, either thrombolysis or PPCI, as the myocardial reperfusion strategy. Interestingly, many of the early failed attempts to reduce myocardial reperfusion injury in the clinical setting were undertaken in the pre-PPCI era with the majority of patients receiving thrombolytic therapy (*Table 1*). Whether a different outcome would have been observed in the setting of PPCI is not known. On the other hand, since PPCI is indeed poorly accessible in most non-Western countries, it is important that cardioprotective interventions be tested using the two different reperfusion strategies in separate studies, To provide potential benefit in the largest possible number of patients worldwide. However, it must be appreciated that myocardial reperfusion by thrombolytic therapy is not the ideal model for investigating the efficacy of novel cardioprotective strategies in STEMI patients because of the issues outlined above.

4.1.3 Timing the therapeutic intervention

Timing the administration of the therapeutic intervention in STEMI patients undergoing myocardial reperfusion using either thrombolytic therapy or PPCI is essential. The detrimental effects of myocardial reperfusion injury occur in the first few minutes of myocardial reperfusion, with pre-clinical animal MI studies demonstrating that unless the study intervention is administered prior to myocardial reperfusion, it is ineffective.[33] The failure to administer the study intervention prior to myocardial reperfusion in some clinical studies may explain in part some of the negative data shown in *Table 1*.

The study treatment may be administered at any time between first patient contact and the time of reperfusion, provided the pharmacokinetics of the drug allow sufficient delivery to the target organ as

Table 2 Clinical studies which have demonstrated beneficial effects in STEMI patients with a therapeutic intervention administered at myocardial reperfusion

Clinical study	Therapeutic intervention	*n*, number	Outcome	Notes
	Atrial natriuretic peptide			
Kitakaze *et al.*, J-WIND-ANP[67]	IV carperitide 72 h infusion started *after* reperfusion	569	15% reduction in 72 h AUC total CK and 2.0% absolute increase in the LVEF	Anterior STEMI only: no Only PPCI or thrombolysis: yes AAR measured: no Collateral flow excluded: no TIMI flow grade <1: no Treatment prior to or at reperfusion: no
	Ischaemic postconditioning			
Staat *et al.*[65]	Four-60 s angioplasty balloon inflations/deflations	30	36%↓ in 72 h AUC CK 34%↓ in peak CK MBG↑ 1.7–2.4	Anterior STEMI only: yes Only PPCI or thrombolysis: yes AAR measured: yes Collateral flow excluded: yes TIMI flow grade <1: yes Treatment prior to or at reperfusion: yes
Thibault *et al.*[85]	Four-60 s angioplasty balloon inflations/deflations	38	41%↓ 72 h AUC CK-MB 39%↓ MI size at 6 months by SPECT 7%↑ EF by echo at 1year	Anterior STEMI only: yes Only PPCI or thrombolysis: yes AAR measured: yes Collateral flow excluded: yes TIMI flow grade <1: yes Treatment prior to or at reperfusion: yes
Lonborg *et al.*[118]	Four-30 s angioplasty balloon inflations/deflations	118	No difference in troponin T or LVEF 19%↓ MI size at 3 months by CMR 31%↑ in the myocardial salvage index Less heart failure	Anterior STEMI only: no Only PPCI or thrombolysis: yes AAR measured: yes Collateral flow excluded: no TIMI flow grade <1: yes Treatment prior to or at reperfusion: yes
Sorensson *et al.*[119]	Four-60 s angioplasty balloon inflations/deflations	76	No difference in 48 h AUC CK-MB/TnT or myocardial salvage by CMR at Day 7–9 Increase in myocardial salvage in patients with large AAR (>30% of LV).	Anterior STEMI only: no Only PPCI or thrombolysis: yes AAR measured: yes Collateral flow excluded: yes TIMI flow grade <1: yes Treatment prior to or at reperfusion: yes
	Cyclosporin A			
Piot *et al.*[68]	IV CsA (2.5 mg/kg) 10 min *prior* to PPCI	58	44%↓MI size (72 h AUC total CK) 20% ↓MI size (CMR in subset of 27 patients) 28% ↓MI size and smaller LVESV on CMR at 6 months[120]	Anterior STEMI only: no Only PPCI or thrombolysis: yes AAR measured: yes Collateral flow excluded: yes TIMI flow grade <1: yes Treatment prior to or at reperfusion: yes
	Therapeutic hypothermia			
Gotberg *et al.*,[63] RAPID-MI-ICE	Cooling by IV infusion of 1–2 L of cold saline and central venous catheter cooling with Philips InnerCool RTx Endovascular System *prior* to PPCI to achieve a core body temperature of 35°C	20	Significant reduction in the MI size as % of AAR on CMR at 4 days 43% reduction in peak and cumulative trop T release	Anterior STEMI only: yes Only PPCI or thrombolysis: yes AAR measured: yes Collateral flow excluded: no TIMI flow grade <1: no Treatment prior to or at reperfusion: Yes
Erlinge *et al.*, CHILL-MI, NCT01379261	Cooling by IV infusion of 1–2 L of cold saline and central venous catheter cooling with Philips InnerCool RTx Endovascular System *prior* to PPCI to achieve a core body temperature of 35°C	120	MI size (as a % of AAR) by CMR at 4 days	Anterior STEMI only: no Only PPCI or thrombolysis: yes AAR measured: yes Collateral flow excluded: no TIMI flow grade <1: no Treatment prior to or at reperfusion: Yes

Continued

Table 2 *Continued*

Clinical study	Therapeutic intervention	*n*, number	Outcome	Notes
	Therapeutic hyperoxaemia			
O'Neill *et al.*,[64] AMIHOT I	IC hyperbaric hyperoxaemic reperfusion started *after* PPCI and continued for 90 min	269	No difference in primary endpoint (14 days MI size by SPECT)	Anterior STEMI only: yes Only PPCI or thrombolysis: yes AAR measured: no Collateral flow excluded: no TIMI flow grade <1: no Treatment prior to or at reperfusion: yes
Stone *et al.*,[121] AMIHOT II	IC hyperbaric hyperoxaemic reperfusion started *after* PPCI and continued for 90 min	281	No adverse events No difference in the MI size by SPECT at 14 days or peak CK-MB or trop. pooled analysis of AMIHOT I and II trials suggested beneficial effects on the MI size and MACE	Anterior STEMI only: yes Only PPCI or thrombolysis: yes AAR measured: no Collateral flow excluded: no TIMI flow grade <1: no Treatment prior to or at reperfusion: yes
	Remote ischaemic conditioning			
Botker *et al.*[66]	Four 5-min inflations/deflations of an upper arm cuff delivered in ambulance by paramedics *prior* to PPCI	142	Increase in the myocardial salvage index at 30 days. No difference in the MI size (SPECT or Peak Trop). Ant STEMI subgroup had greater myocardial salvage, smaller MI size, and better LV function at 3 days[122]	Anterior STEMI only: no Only PPCI or thrombolysis: yes AAR measured: yes Collateral flow excluded: no TIMI flow grade <1: no Treatment prior to or at reperfusion: yes
Rentoukas *et al.*[123]	Three-4 min inflations/deflations of an upper arm cuff delivered on arrival at the hospital *prior* to PPCI	93	Better ST resolution and lower peak Trop I. Synergistic effects with morphine	Anterior STEMI only: no Only PPCI or thrombolysis: yes AAR measured: no Collateral flow excluded: no TIMI flow grade <1: no Treatment prior to or at reperfusion: yes
	Exenatide			
Lonborg *et al.*[69]	IV infusion of exenatide started 15 min *prior* to PPCI for 6 h	107	Increase in the myocardial salvage index at 90 days by CMR. Reduced MI size as % of AAR at 90 days by CMR. Patients presenting with short ischaemic times (≤132 min) had greater myocardial salvage[124]	Anterior STEMI only: no Only PPCI or thrombolysis: yes AAR measured: yes Collateral flow excluded: no TIMI flow grade <1: yes Treatment prior to or at reperfusion: yes
	Glucose insulin potassium (GIK) therapy			
Selker *et al.*, IMMEDIATE[72]	Iv GIK infusion for 12 h started by paramedics in ambulance—*prior* to reperfusion	357	No difference in progression to MI Reduction in the MI size and less in-hospital mortality and cardiac arrest	Anterior STEMI only: no Only PPCI or thrombolysis: yes AAR measured: no Collateral flow excluded: no TIMI flow grade <1: no Treatment prior to or at reperfusion: yes

Anterior STEMI only, Only anterior STEMI patients included, Only PPCI or thrombolysis: Only either PPCI or thrombolysis patients included, AAR measured: Area at risk measured; Collateral flow excluded: Coronary collateralization to the AAR excluded; TIMI flow grade <1, TIMI flow grade less than 1 in the infarct-related artery prior to PCI; Treatment prior to or at reperfusion, Study intervention given prior to or at reperfusion.

soon as the myocardial blood flow is turned on again. This may explain the negative results observed in clinical studies investigating oral atorvastatin as a cardioprotective intervention in STEMI patients (see *Table 1*). The study intervention may, for example, be administered in the ambulance to the suspected STEMI patient while in transit to the hospital. This therapeutic approach has been employed with GIK therapy[72] and RIC,[66] and is currently being investigated for metoprolol therapy. However, one specific limitation of this treatment strategy is that a significant proportion of suspected STEMI patients (perhaps 20–30%) will end up not having a diagnosis of STEMI, and will have therefore received the therapeutic intervention un-necessarily. The same problem applies to administering the cardioprotective strategy on immediate arrival at the hospital. One potential approach for selecting STEMI patients is to only select patients for study after coronary angiography has taken place. This approach will also allow one to exclude those patients with TIMI>1

Table 3 Clinical studies investigating therapeutic interventions administered at myocardial reperfusion which have potential promise in STEMI patients

Clinical study	Therapeutic intervention	*n*, number	Outcome	Notes
	Adenosine			
Garcia-Dorado *et al.*,[60] PROMISE	Intracoronary adenosine 4 mg *prior* to PPCI	201	MI size on CMR at 5–10 days. Ongoing study	Anterior STEMI only: no Only PPCI or thrombolysis: yes AAR measured: yes Collateral flow excluded: no TIMI flow grade <1: no Treatment prior to or at reperfusion: yes
	Bendavia (MTP)			
Chakrabarti *et al.*, EMBRACE, NCT01572909	Bendavia at time of PPCI.	200	Primary endpoint is the MI size (72 h AUC CK-MB)	Anterior STEMI only: yes Only PPCI or thrombolysis: yes AAR measured: no Collateral flow excluded: no TIMI flow grade <1: no Treatment prior to or at reperfusion: yes
	Impella 2.5			
Moses *et al.*, MINI-AMI, NCT01319760	Impella 2.5 *after* PPCI for 24 h	50	Primary endpoint is the MI size at 3–5 days by CMR	Anterior STEMI only: yes Only PPCI or thrombolysis: yes AAR measured: no Collateral flow excluded: no TIMI flow grade <1: no Treatment prior to or at reperfusion: yes
	Insulin-like growth factor-1			
Caplice *et al.*, RESUS-AMI, NCT01438086	Intracoronary rhIGF-1 (mecasermin)	45	Serum glucose and change in the LVEF on CMR	Anterior STEMI only: no Only PPCI or thrombolysis: yes AAR measured: no Collateral flow excluded: no TIMI flow grade <1: no Treatment prior to or at reperfusion: yes
	Mangafodipir (Teslascan)			
Karlsson *et al.*, MANAMI, NCT00966563	Iv infusion over 2–5 min *prior* to PPCI	20	The primary endpoint is the MI size (Trop T/CK-MB)	Anterior STEMI only: no Only PPCI or thrombolysis: yes AAR measured: no Collateral flow excluded: no TIMI flow grade <1: no Treatment prior to or at reperfusion: yes
	Melatonin			
Dominguez-Rodrigeuz *et al.*, MARIA, NCT00640094	Iv infusion at time of PPCI	272	The primary endpoint is the MI size (72 h AUC alpha-hydroxybutyrate dehydrogenase)	Anterior STEMI only: no Only PPCI or thrombolysis: yes AAR measured: no Collateral flow excluded: no TIMI flow grade <1: no Treatment prior to or at reperfusion: yes
Halladin *et al.*, NCT01172171	Intracoronary and iv infusion at time of PPCI	60	The primary endpoint is the MI size (CMR at 1 month)	Anterior STEMI only: yes Only PPCI or thrombolysis: yes AAR measured: no Collateral flow excluded: no TIMI flow grade <1: no Treatment prior to or at reperfusion: yes
	Nitric oxide (inhaled)			

Continued

Table 3 *Continued*

Clinical study	Therapeutic intervention	*n*, number	Outcome	Notes
Janssens *et al*., NOMI, NCT01398384	Inhaled nitric oxide *prior* to PPCI	230	The primary endpoint is the MI size as a % of LV at 3 days by CMR	Anterior STEMI only: no Only PPCI or thrombolysis: yes AAR measured: no Collateral flow excluded: no TIMI flow grade <1: yes Treatment prior to or at reperfusion: yes
	Nitrite (sodium)			
Frennaux *et al*., NIAMI, NCT01388504	Iv bolus of sodium nitrite given 5 min *prior* to PPCI	200	The primary endpoint is the MI size as a % of AAR at 10–14 days by CMR	Anterior STEMI only: no Only PPCI or thrombolysis: yes AAR measured: no Collateral flow excluded: no TIMI flow grade <1: yes Treatment prior to or at reperfusion: yes
Mathur *et al*., NITRITE-AMI, NCT01584453	Intracoronary bolus of sodium nitrite over 30–60 s at the time of PPCI	80	The primary endpoint is the MI size (48 h Trop T AUC)	Anterior STEMI only: no Only PPCI or thrombolysis: yes AAR measured: no Collateral flow excluded: no TIMI flow grade <1: no Treatment prior to or at reperfusion: yes
	RIC and local IPost			
Prunier *et al*., RIRE-1, NCT01390142	Four 5-min inflations/deflations of the upper arm cuff *prior* to PPCI plus four-1min inflations/ deflations of angioplasty balloon after PPCI	50	The primary endpoint is the MI size (72 h CK-MB AUC) and MI size at 3 months (CMR)	Anterior STEMI only: no Only PPCI or thrombolysis: yes AAR measured: no Collateral flow excluded: no TIMI flow grade <1: no Treatment prior to or at reperfusion: yes
	Sevoflurane			
Lavi *et al*., SIAMI, NCT00971607	Inhaled sevoflurane *during* PPCI	50	The primary endpoint is the MI size (serum biomarkers over 72 h).	Anterior STEMI only: no Only PPCI or thrombolysis: yes AAR measured: no Collateral flow excluded: no TIMI flow grade <1: no Treatment prior to or at reperfusion: yes
	Thymosin Beta 4			
Strobeck *et al*., NCT00378352	Iv injection of RGN-352 (Thymosin Beta 4)	75	The primary endpoint is the MI size on CMR at 28 days	Anterior STEMI only: yes Only PPCI or thrombolysis: yes AAR measured: no Collateral flow excluded: no TIMI flow grade <1: no Treatment prior to or at reperfusion: yes
	TRO40303			
Atar *et al*., MitoCare, NCT01374321	Peripheral IV infusion of TRO40303 started at 5–15 min *prior* to PPCI	180	MI size (72 h AUC CK and Trop I)	Anterior STEMI only: no Only PPCI or thrombolysis: yes AAR measured: no Collateral flow excluded: no TIMI flow grade <1: yes Treatment prior to or at reperfusion: yes
	Metoprolol			

Continued

Table 3 *Continued*

Clinical study	Therapeutic intervention	*n*, number	Outcome	Notes
Ibanez *et al.*, METOCARD-CNIC, NCT01311700	Iv metoprolol three-5 mg boluses administered in ambulance *prior* to PPCI	220	MI size (5–7 days by CMR)	Anterior STEMI only: no Only PPCI or thrombolysis: yes AAR measured: no Collateral flow excluded: no TIMI flow grade <1: yes Treatment prior to or at reperfusion: yes

Anterior STEMI only, only anterior STEMI patients included; only PPCI or thrombolysis, only either PPCI or thrombolysis patients included; AAR measured, area at risk measured; collateral flow excluded, coronary collateralization to the AAR excluded; TIMI flow grade <1, TIMI flow grade <1 in the infarct-related artery prior to PCI; treatment prior to or at reperfusion, study intervention given prior to or at reperfusion.

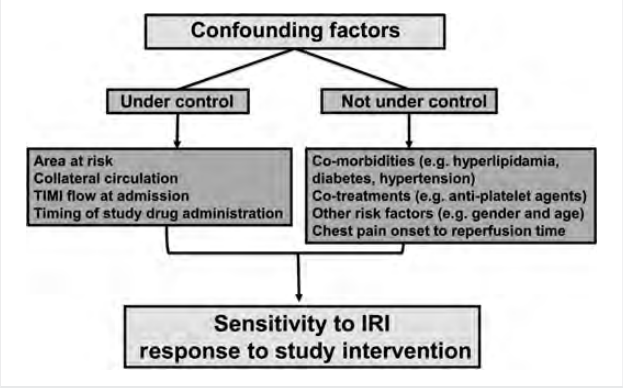

Figure 1 Summary of confounding factors which impact on the sensitivity to ischaemia–reperfusion injury (IRI) and the response to the study intervention in STEMI patients. These can be divided into those factors which can be controlled for and those factors which cannot be controlled for when designing a clinical cardioprotection STEMI study.

coronary flow in the infarct-related artery and significant coronary collateralization to the area at risk (AAR) (Rentrop grade>1). The obvious disadvantage of waiting until coronary angiography has taken place is the limited time remaining to then administer the therapeutic intervention before myocardial reperfusion takes place. Pharmacological study interventions should be administered either using the iv or intracoronary route to ensure that therapeutic concentrations of the study agent are achieved prior to myocardial reperfusion. The intracoronary route will achieve higher local concentrations within the myocardium, which may allow a lower dose of the drug to be used should the pharmacological agent have systemic hemodynamic effects. In our future daily practice, it is likely that the optimal timing of administration of a proven protective drug will have to comply with its modalities of administration and its pharmacokinetics.

4.1.4 Major determinants of MI size

For clinical cardioprotection trials investigating the MI-limiting effects of a study intervention, it is essential to assess for the major determinants of MI size in STEMI patients undergoing myocardial reperfusion.

4.1.4.1 Ischaemic time

The duration of acute myocardial ischaemia is a major determinant of final MI size. In pre-clinical animal MI studies investigating novel therapeutic interventions the ischaemic time can obviously be chosen to generate a relatively fixed MI size. However, in STEMI patients, the ischaemic time can vary between 0 to 12 h, depending on the chest pain onset to reperfusion time, resulting in widely variable MI sizes. Myocardial reperfusion accrues the most benefit in terms of myocardial salvage in those patients presenting within 3 h of chest pain onset. Whether MI size reduction with a study intervention is greater in patients presenting early (within 3 h) or later (3 h and beyond) is not clear. Two clinical studies have reported greater benefit with the pharmacological agents adenosine or exenatide in terms of myocardial salvage in patients presenting within 2–3 h of chest pain onset, suggesting that the former may be true (see *Table 2*). Whether IPost or RIC is more beneficial when administered to patients presenting with shorter or longer ischaemic times is not clear. One pre-clinical study suggests that IPost was actually harmful if applied following a short episode of index ischaemia,[77] suggesting that IPost may be more beneficial in patients with longer ischaemic times. Moreover, laboratory studies suggest the relative importance of different mechanisms of reperfusion injury may depend on the duration of ischaemia, with mitochondrial permeability transition playing a more prominent role after prolonged ischaemia.[78]

However, it is important to consider that the benefit obtained in terms of myocardial salvage does not necessarily result in patient benefit expressed in terms of clinical outcomes. Although myocardial salvage following a protective intervention may be greater in patients reperfused within the first 3 h of onset of symptoms it is most likely related to a reduction of the ischaemic damage that has developed rapidly in the first hours of ischaemia. However, these patients usually display small infarcts with good clinical prognosis, so that the improved myocardial salvage may even not be clinically visible. On the other end of the spectrum, one cannot exclude that even mild myocardial salvage in patients with a prolonged (>6 h) ischaemic insult may translate into a significant clinical benefit, including limitation of adverse LV remodelling for example. Additional studies are required to actually understand the impact of the ischaemia time on IRI and clinical outcome.

4.1.4.2 The area at risk

The size of the AAR is a major determinant of the final MI size.[79] Because of this, it is essential to take into account the size of the

AAR when assessing MI size reduction with novel therapeutic interventions in clinical studies. The ability to measure the AAR is particularly important in STEMI patients, where the size of the AAR can vary greatly (from 10 to 50% of the LV) depending on which the coronary artery is involved (LAD, RCA, or Cx) and where along the vessel the occlusion has occurred (proximal, mid-vessel, or distal).[80] However, in most clinical cardioprotection STEMI trials, the size of the AAR is not measured. In clinical studies, the MI size can be expressed as a percentage of the size of the AAR in a similar manner to the preclinical studies. However, the more conventional approach is to calculate the myocardial salvage index (MSI, which is defined as the size of the AAR subtract MI size divided by size of the AAR).

There are several different techniques available for estimating the size of the AAR and calculating the MSI in STEMI patients undergoing myocardial reperfusion (Supplementary material online, *Table S3*).[81] The current gold standard method for measuring AAR is ^{99m}Technetium-Sestamibi single-photon emission tomography (SPECT), with cardiac MRI emerging as a potential alternative approach, although both these imaging techniques have their drawbacks (Supplementary material online, *Table S3*).[58,82,83] With respect to the potential use of cardiac MRI for delineating the AAR in STEMI patients undergoing PPCI, the field is particularly controversial with several issues of concern including the technical limitations surrounding T2-weighted imaging[84] and the possibility of the cardioprotective intervention reducing the size of the AAR by decreasing the extent of myocardial oedema.[83]

Previous clinical cardioprotection studies in STEMI patients suggest that the patients most likely to benefit from a study intervention administered as an adjunct to myocardial reperfusion are those presenting with a large AAR (>30% of the LV—usually proximal LAD and RCA STEMI patients).[65,66,68,69,85] Therefore, in those clinical studies in which all STEMI patients were included, irrespective of the size of the AAR, there is a possibility that any cardioprotective effect associated with the study intervention is diluted and this may account in part for some of the negative clinical cardioprotection studies in which all-STEMI patients were included (*Table 1*).[86] Therefore, it is essential that the AAR is measured when designing a clinical cardioprotection study comprising STEMI patients.

4.1.4.3 Coronary collateralization to the area at risk

The presence of coronary collateralization to the AAR may provide some residual blood flow to the ischaemic bed after a coronary artery occlusion and reduce MI size.[87] Therefore, collateral flow needs to be measured in clinical studies when investigating MI-reduction study interventions. About 15–20% of STEMI patients will have significant coronary collateralization to the AAR.[58] These patients sustain smaller myocardial infarcts and have better clinical outcomes, when compared with those patients with little collateralization. However, measuring collateral flow reliably in patients presenting with a STEMI is challenging. At the time of coronary angiography, the Rentrop grading system can be used to assess whether significant coronary collateralization to the AAR is present, and these patients should therefore be excluded from clinical cardioprotection studies of STEMI patients, as they are less likely to benefit from a study intervention. Including patients with significant coronary collateralization to the AAR may in part explain the negative findings of the clinical cardioprotection studies listed in *Table 1*.

4.1.4.4 Coronary artery flow prior to myocardial reperfusion

Because modern efficient anti-platelet and anti-thrombotic therapies are instituted early, >40% of STEMI patients presenting to the hospital will have spontaneously reperfused prior to PPCI and will already have a significant coronary flow (TIMI flow >1) within the culprit coronary artery.[66] For these patients, in whom myocardial reperfusion has already taken place, the prognosis is improved when compared with those patients presenting with a fully occluded culprit artery. For the study intervention to be effective against myocardial reperfusion injury, it needs to be administered to the STEMI patient while the culprit artery is still occluded and prior to myocardial reperfusion. This would explain why STEMI patients presenting with an occluded culprit artery accrued the most benefit in terms of MI size reduction with RIC.[66] On this basis, for clinical cardioprotection studies, it is advisable to only include those STEMI patients with an occluded culprit artery.

One may question whether these four major confounding factors ought to be measured in clinical outcome studies. Here, the endpoint is not MI size, but rather death, or hospitalization for heart failure for example. In these trials, it remains essential not to include patients who display a spontaneously re-opened coronary artery on admission coronary angiography, since they have already undergone myocardial reperfusion injury (before the protective intervention could be administered). Not considering patients with visible collaterals should also be recommended, as mentioned above. But, whether AAR is a strong predictor of such clinical events remains to be demonstrated and the recruitment of a large number of patients together with the randomization process will balance between the placebo and the active treatment group the distribution of ischaemia time and sizes of AAR. Where it is difficult to perform such a large clinical study one may consider stratifying the study with respect to the AAR. One might yet want to include patients with large AAR (e.g. anterior infarcts) who constitute the high-risk population which would benefit the most, in terms of clinical outcome, of cardioprotective interventions.

4.1.4.5 Endpoints of cardioprotection

For a clinical cardioprotection study in STEMI patients, it is essential to choose study endpoints which are most relevant to the MI size limiting effects of the study intervention being investigated. For proof-of-concept clinical studies, this will most likely include endpoints of cardioprotection such as MI size (using either 48 h AUC cardiac troponins or late gadolinium enhancement on cardiac MRI), left ventricular systolic function, and indexed left ventricular volumes. If the AAR is measured in the clinical study, then MI size should take into account the size of the AAR, which will increase the statistical power of the clinical trial for detecting a significant reduction in MI size, thereby reducing the number of patients required for the study.

In terms of designing the larger clinical outcome studies, it is crucial to choose major adverse cardiac events (MACEs) which are relevant to the MI size limiting effects of the study intervention. In this regard, the combined rates of cardiac death and hospitalization for heart failure are most relevant to MI size limitation in STEMI patients as a combined primary study endpoint, whereas rates of coronary revascularization and non-fatal MI are less relevant and unlikely to be influenced by a MI size limiting study intervention.

4.2 Confounding factors which cannot be controlled for

In CHD patients, there are a large number of confounding factors which can potentially alter the sensitivity of the heart to acute IRI and/or interfere with the efficacy of a particular cardioprotective study intervention. A major cause of these confounding factors relates to the fact that CHD is caused by or associated with known cardiovascular risk factors and co-morbidities, including ageing, hypertension, hyperlipidaemia, diabetes, left ventricular hypertrophy, heart failure, and uraemia.[88] Pre-clinical animal studies suggest that these diseases and their pharmacological treatments induce fundamental molecular alterations in the heart that can potentially affect the cytoprotective signalling pathways, thereby affecting both the sensitivity to IRI and the response to a particular cardioprotection strategy (reviewed in[88,89]). Currently, most of the animal MI models which are used to assess the efficacy of a novel cardioprotective strategy use healthy juvenile animals which are free of any co-morbid disease.[1] Furthermore, pre-clinical studies have reported that human atrial tissue harvested at time of cardiac bypass surgery from aged patients[90] and diabetic patients,[91,92] were resistant to IPC applied *ex vivo* in a model of simulated IRI. Large-scale cohorts of STEMI patients are needed to analyse how much age, gender, co-morbidities, and co-treatments may affect IRI and response to protective interventions. In addition, it has to be taken into account that most patients display several comorbidities. Specific analyses will then help adapt future therapies to specific subgroups of patients.

Another major confounding factor for cardioprotection is concomitant medication, which patients are on for their cardiovascular risk factor, co-morbid condition, or as part of the treatment of the ongoing acute MI. These pharmaceutical agents have been shown in pre-clinical and clinical studies to either block the cardioprotective effect (for example, certain oral anti-diabetic sulphonylureas, nitrates when nitrate tolerance develops, certain statins) or induce cardioprotection themselves (for example, insulin and some anti-diabetic medications, some statins, ACE-inhibitors, anti-platelet agents, volatile anaesthetic agents, opioids, and so on (reviewed in[4,88,89,93–95]). So far, the animal MI models which are used to assess the efficacy of a novel cardioprotective strategy do not investigate the effect of concomitant medication. Treatment with oral sulphonylureas such as glibenclamide[96] and the anti-anginal agent, nicorandil,[97] also interferes with IPC protection in human atrial tissue. With respect to concomitant anti-platelet therapy, there is preliminary animal data suggesting that these agents may actually confer direct protection against acute IRI,[98,99] an observation which is likely to have a significant impact on clinical cardioprotection studies, as most CHD patients will be on anti-platelet therapy.

Whether these confounding factors can actually interfere with the efficacy of a cardioprotective intervention or an endogenous cardioprotective phenomenon in the clinical setting has only been investigated directly in relatively few clinical studies (for a summary of the major clinical studies, see Supplementary material online, *Table S4*). None of these confounding factors was pre-specified but all were investigated as retrospective *post hoc* subgroup analyses. In a recent retrospective analysis of proof-of-concept IPost trials, Roubille *et al.*[93] reported that clopidogrel administered before PCI may indeed be a confounder both for sensitivity to IRI and response to angioplasty postconditioning in STEMI patients. This may in part explain some of the recently published clinical studies which failed to demonstrate any cardioprotective effect with IPost.[100,101]

5. Confounding factors in CABG cardioprotection studies

A number of different therapeutic interventions have been investigated in the setting of CABG and major vascular surgery, with many of them failing to report any beneficial effects on peri-operative myocardial injury/infarction or clinical outcomes (*Table 4*). Recently, several proof-of-concept clinical trials have reported cardioprotective effects with therapeutic strategies including RIC, IPost, glucose–insulin–potassium (GIK) therapy and volatile anaesthetics (*Table 5*). However, even with RIC, not all clinical studies have been positive, an issue which is discussed in a later section.[102] Whether RIC can improve clinical outcomes in patients undergoing CABG surgery is unknown, and is currently being investigated in two ongoing large multicentre randomized clinical trials such as the ERICCA (NCT01247545)[103] and RIPHeart trials (NCT01067703).[104] Large multicentre clinical trials are required to investigate whether GIK or volatile anaesthetics can improve clinical outcomes in patients undergoing CABG surgery.

5.1 Patient selection

The heterogeneity of patients undergoing cardiopulmonary bypass surgery and the number of confounding factors which can potentially interfere with cardioprotection make patient selection for clinical cardioprotection studies in CABG patients quite challenging. The type of surgery is clearly important, with patients undergoing CABG alone very different from patients undergoing valve surgery. For example, the myocardium of patients with severe aortic stenosis or aortic regurgitation may be significantly hypertrophied, the presence of which may impact on the cardioprotective effect of a study intervention. For patients undergoing CABG surgery, it must be acknowledged that direct injury to the myocardium during surgery can happen and may contribute to cardiac enzyme release; this has to be taken into account when interpreting the results. Therefore, it may be advisable to select patients undergoing either CABG alone or valve surgery alone in a clinical cardioprotection study.

Of course the type of surgery will impact on the aortic cross-clamp time (the duration of acute global myocardial ischaemia) with longer cross-clamp times in patients undergoing more complex surgery (for example, CABG+valve surgery). This would be another reason for separating CABG alone patients from valve surgery patients in clinical cardioprotection studies.

Whether a patient has stable CHD or unstable CHD may also affect the response of the myocardium to the study intervention. Unstable patients may be sicker and have experienced episodes of chest pain at rest, which may have inadvertently preconditioned the myocardium against acute IRI.[105] Therefore, for clinical cardioprotection studies it would be advisable to recruit either stable or unstable patients. Specifically, it is important in either stable or unstable patients to exclude patients who have experienced anginal symptoms in the 24 h prior to surgery, to exclude the confounding effect of IPC.

Table 4 Clinical studies which have failed to demonstrate any beneficial effect in CABG patients with a cardioprotective intervention

Clinical study	Therapeutic intervention	*n*, number	Primary outcome	Notes
	Sodium-hydrogen ion exchange inhibitors			
Boyce *et al.*, GUARDIAN[125]	Oral cariporide or placebo prior to surgery	1477, CABG	Less CK-MB release 25% ↓risk of death and non-fatal MI at 36 days Beneficial effect maintained at 6 months post-surgery	
Mentzer *et al.*, EXPEDITION[126]	Oral cariporide or placebo prior to surgery	5761, CABG	Reduction in the primary endpoint of death and MI However, increase in mortality due to cerebrovascular events	Off-target cerebral effects
	Acadesine			
Mangano *et al.*[127]	Acadesine given as IV infusion and in the cardioplegic solution	2695, CABG	No difference in the primary endpoint of cardiac death, MI, or stroke at 4 days	No difference in peri-operative MI (PMI), but in the 100 patients who did have a PMI (3.7%) patients, acadesine reduced patient death
Newman *et al.*, RED-CABG[128]	Acadesine given as IV infusion and in cardioplegic solution	3080, CABG	No difference in the primary endpoint of all-cause mortality, non-fatal stroke, or severe left ventricular dysfunction at Day 28	Trial stopped early because of futility analysis indicating a very low likelihood of a statistically significant efficacious outcome
	Pexelizumab			
Verrier *et al.*, PRIMO-CABG[129]	IV pexelizumab bolus prior to CABG followed by 24 h infusion	2476, CABG	Non-significant reduction in the primary combined 30-day endpoint of death and non-fatal MI	Only targets the anti-inflammatory component of acute IRI
Smith *et al.*, PRIMO-CABG2[130]	IV pexelizumab bolus prior to CABG followed by 24 h infusion	4254, CABG	No difference in the primary combined 30-day endpoint of death and non-fatal MI	Only targets the anti-inflammatory component of acute IRI

MI, myocardial infarction; TnI, Troponin I; TnT, Troponin T; h, hours.

5.2 Peri-operative factors

5.2.1 Concomitant medication

A wide variety of pharmacological agents used during cardiopulmonary bypass surgery may interfere with the cardioprotective efficacy of a study intervention. Volatile anaesthetic agents (such as isoflurane and sevoflurane) and the iv anaesthetic agent, propofol, have been reported to either confer cardioprotection themselves or interfere with RIC cardioprotection (see *Table 5*). Furthermore, the use of iv GTN, nitroprusside, and opioid analgesics may also interfere with the cardioprotective effects of a study intervention. However, it may be difficult to standardize the anaesthetic regimen and concomitant medication in clinical cardioprotection studies given the variations in practice. Providing the study is adequately powered and properly randomized these confounding factors should distribute themselves equally between the study intervention and control treatment groups. Where it is difficult to perform such a large clinical study one may consider stratifying the study with respect to these confounding factors.

5.2.2 Myocardial preservation strategy

During cardiopulmonary bypass surgery, it is essential to create a blood-free and motionless operative field, in order to improve visibility, facilitate the surgical procedure and prevent air-embolism. This is achieved by cross-clamping the aorta (to isolate the heart from the systemic circulation) and inducing electrochemical cardiac arrest (to stop the heart beating) using cardioplegic solution, respectively. The choice of myocardial preservation strategy (blood cardioplegia, crystalloid cardioplegia, or cross-clamp fibrillation) may impact on the cardioprotective efficacy of the study agent, but again providing the study is adequately powered and properly randomized this should not be a major issue.

5.3 The therapeutic intervention

In clinical cardioprotection studies in cardiopulmonary surgery, there is the opportunity to apply the study intervention at several different time-points: either before CABG surgery begins (prior to acute myocardial ischaemia), by adding a pharmacological intervention to the cardioplegic solution after aortic cross-clamping (after the onset of acute myocardial ischaemia), or at the time of aortic declamping (at the time of myocardial reperfusion). It is important to ensure that the time elapsed between administering the preconditioning study intervention and the time of aortic declamping (acute myocardial reperfusion injury) does not exceed the 2–3 h, as this corresponds to the cardioprotective window of protection elicited by IPC.

5.4 Endpoints to assess cardioprotection

For a clinical cardioprotection study in CABG patients, it is essential to choose study endpoints which are most relevant to the cardioprotective effects of the study intervention being investigated. For

Table 5 Clinical studies investigating therapeutic interventions which have shown benefit in the setting of cardiac bypass or major vascular surgery

Clinical study	Therapeutic intervention	*n*, number, surgery	Primary outcome	Notes
Positive studies	*Remote ischaemic conditioning*			
Cheung *et al.*[131]	Four 5-min inflations/deflations of thigh cuff	37 children, CHD	43% reduction in 72 h AUC TnT	Reduced ventilation time and inotrope requirements
Hausenloy *et al.*[132]	Three 5-min inflations/deflations of the upper arm cuff	58 adults, CABG	43% reduction in 72 h AUC TnT	
Ali *et al.*[133]	Clamping of the right common iliac artery for 10 min followed by clamping of the left common iliac artery for 10 min	82 adults, AAA	98% reduction in 7 days AUC Trop I	Less acute kidney injury
Venugopal *et al.*[134]	Three 5-min inflations/deflations of the upper arm cuff	45 adults, CABG	42% reduction in 72 h AUC TnT	
Wagner *et al.*[135]	Three 5-min inflations/deflations of the upper arm cuff 18 h prior to surgery	67 adults, CABG+AVR	Reduction in peak Trop-I at 8 h	First demonstration of delayed RIC in this clinical setting St Thomas' crystalloid cardioplegia
Thielmann *et al.*[136]	Three 5-min inflations/deflations of the upper arm cuff	53 adults, CABG	45% reduction in 72 h AUC TnI	Bretschneider crystalloid cardioplegia used
Li *et al.*[137]	Three 5-min inflations/deflations of the upper arm cuff after aortic cross-clamp	82, valve surgery only	Reduction in peak TnT at 30 min	First demonstration of delayed RIC in this clinical setting However, when RIC protocol delivered prior to CABG surgery, no difference in peak Trop T
Choi *et al.*[138]	Three 10-min inflations/deflations of thigh arm cuff	76, valve surgery	Reduction in peak CK-MB at 24 h	
Wu *et al.*[139]	Three 5-min inflations/deflations of the upper arm cuff with two-10 min inflations/deflations of thigh cuff	75, MVR	Reduced peak TnI at 6, 12, 24, 48, 72 h	RIC of arm did not reduce TnI First demonstration that combining arm and leg RIC more effective that arm alone
Kottenberg *et al.*[140]	Three 5-min inflations/deflations of the upper arm cuff	72 adults, CABG	50% reduction in 72 h AUC TnI with Isoflurane but not Propofol.	Bretschneider crystalloid cardioplegia used
Xie *et al.*[141]	Three 5-min inflations/deflations of the upper arm cuff	73 adults, valve surgery	43% reduction in 72 h AUC TnI	
Heusch *et al.*[18]	Three 5-min inflations/deflations of the upper arm cuff	23 adults, CABG	A significant reduction in 72 h AUC TnI	Bretschneider crystalloid cardioplegia used
Negative studies	*Remote ischaemic conditioning*			
Rahman *et al.*[142]	Three 5-min inflations/deflations of the upper arm cuff	162 adults, CABG	No difference in 48 h AUC TnT	RIC protocol administered at the time of the surgical incision IV GTN given to all patients Sevoflurane and propofol both used for maintenance anaesthesia
Li *et al.*[137]	Three 5-min inflations/deflations of the upper arm cuff	82, valve surgery only	No difference in peak TnT at 30 min	Isoflurane and propofol both used for maintenance anaesthesia However, when RIC protocol delivered after aortic cross-clamp there was a significant reduction in peak Trop T
Karuppasamy *et al.*[143]	Three 5-min inflations/deflations of the upper arm cuff	53 adults, CABG	No difference in 48 h AUC TnI or CK-MB	Isoflurane and propofol both used for maintenance anaesthesia
Wu *et al.*[139]	Three 5-min inflations/deflations of the upper arm cuff	75, MVR	No different in peak Trop I	RIC did not reduce TnI unless except for combined arm and leg RIC
Luchinetti *et al.*[144]	Four 5-min inflations/deflations of thigh cuff	57, CABG	No difference in hsTnT	Propofol used during induction and isoflurane used for maintenance anaesthesia
Young *et al.*[145]	Three 5-min inflations/deflations of the upper arm cuff	96 adults, CABG	Higher plasma levels of hsTnT at 6 and 12 h with RIC	RIC protocol administered at the time of surgical incision Isoflurane and propofol both used for maintenance anaesthesia

Continued

Table 5 *Continued*

Clinical study	Therapeutic intervention	*n*, number, surgery	Primary outcome	Notes
Lomivorotov *et al.*[146]	Three 5-min inflations/deflations of the upper arm cuff	80 adults, CABG	No difference in Trop I or CK-MB	Isoflurane and propofol both used for maintenance anaesthesia Improved cardiac index
	Ischaemic postconditioning			
Luo *et al.*[147]	2 × 30 s cycles of aortic cross-clamping	24 children, CHD	Reduction in TnI and CK-MB at 4 h post-IPost	First study to show efficacy with IPost in cardiac bypass surgery
Luo *et al.*[148]	3 × 30 s cycles of aortic cross-clamping	50 adults, AVR	Reduction in CK-MB but not TnI	IPost also resulted in a reduction in inotrope requirement
Li *et al.*[149]	2 × 30 s cycles of aortic cross-clamping	99 children, CHD	Reduction in TnI at 4 h post-IPost	IPost also resulted in a 44% reduction in ventilation time and 40% reduction in inotrope requirement
	Glucose insulin potassium (GIK) therapy			
Ranasinghe *et al.*[150]	Iv GIK given prior to CABG surgery until 6 h after aortic clamp removal plus or minus IV T3 (given on removal of aortic clamp for 6 h)	440 adults, CABG	Both GIK and T3 therapy increased cardiac index, and reduced TnI at 6 and 12 h	
	Inhaled anaesthetics			
Symons and Myles[151], Meta-analysis of 27 studies	Isoflurane, sevoflurane, desflurane and enflurane	2979, CABG	Better LV function Less inotropes Lower Trop I levels	Shorter duration of mechanical ventilation Shorter hospital stay
Yu and Beattie[152], Meta-analysis of 32 studies	Sevoflurane, desflurane	2841, CABG	Less TnI at 6, 12, 24, and 48 h	
Landoni *et al.*[153], Meta-analysis of 21 studies	Sevoflurane, desflurane	1922, CABG	Decreased incidence and magnitude of PMI Shorter ITU and hospital stay	Less inotrope and ventilation requirements A reduction in mortality

AVR, aortic valve replacement; CHD, congenital heart disease surgery; PMI, peri-operative MI; MI, myocardial infarction; MVR, mitral valve replacement, TnI, Troponin I; TnT, Troponin T; h, hours.

proof-of-concept clinical studies, this will most likely include surrogate endpoints of cardioprotection such as the magnitude of peri-operative myocardial injury (using 72 h AUC cardiac troponins), inotrope requirements, and left ventricular systolic function (which should be measured both acutely and also after 3–4 months to allow for any recovery of LV contractile function from the effects of CABG surgery). In the recently published 'Third Universal Definition of Myocardial Infarction',[45] MI associated with CABG has been arbitrarily defined as an elevation of cardiac biomarker values $>10 \times$ 99th percentile URL in patients with normal baseline Troponin values ($<$99th percentile URL), associated with either (i) new pathological Q-waves or new LBBB or (ii) angiographic documented new graft or new native coronary artery occlusion or (iii) imaging evidence of new loss of viable myocardium or new regional wall motion abnormality.

In CABG patients, where vital organs other than the heart are also subjected to acute IRI, there is an opportunity to investigate whether the study intervention can also confer protection against the detrimental effects of IRI in the lung (measure the ventilation time), brain (assess cognitive function), and kidney (incidence of acute kidney injury).

Once the proof-of-concept clinical study has demonstrated a beneficial effect with a particular study intervention with respect to surrogate endpoints of cardioprotection, the next objective is to determine whether the study intervention can actually improve clinical outcomes in cardiopulmonary bypass surgery patients as evidenced by reducing MACEs. When designing the larger clinical outcome studies, it is preferable to choose MACEs which are most relevant to the cardioprotective effects of the study intervention. Combined rates of cardiac death and hospitalization for heart failure may be the most relevant for CABG patients as a combined primary study endpoint, whereas rates of coronary revascularization, non-fatal MI, and stroke may be less relevant and unlikely to be influenced by a cardioprotective intervention, but these data can be used to assess the safety of the study intervention.

6. Summary and conclusions

Previous attempts to protect the heart against the detrimental effects of acute IRI in patients with CHD have been largely disappointing. One major contributing factor for this failure to translate cardioprotective interventions discovered in animal studies into the clinical setting can be attributed to problems with the clinical study design. In this ESC Working Group Cellular Biology of the Heart Position Paper, we provide recommendations to help optimize the design of clinical cardioprotection studies in STEMI and CABG patients (see *Tables* 6 and 7), which take into account the experience from previously published pre-clinical and clinical data. The hope would be to improve the translation of cardioprotective strategies into the clinical setting for the benefit of CHD patients.

Table 6 Recommendations for designing MI-limiting studies in STEMI patients

Patient selection

- Select patients with a large area at risk (AAR) (>30% of the left ventricle)
- Select patients with no significant coronary collateralization to the AAR (Rentrop grade <1)
- Select patients with an occluded culprit artery at the time of study intervention administration (TIMI flow grade 0 or 1)

The study intervention

- Select a study intervention which has shown conclusive cardioprotection in pre-clinical studies
- Administer the study intervention as an iv or intracoronary bolus *prior* to myocardial reperfusion

Choose MI-limiting-related study endpoints

- MI size (48 h AUC cardiac enzymes or late gadolinium enhancement cardiac MRI)
- Myocardial salvage index (AAR-MI size/AAR)
- Incidence and extent of microvascular obstruction (cardiac MRI)
- Indexed left ventricular end systolic/diastolic dimensions (echocardiography or cardiac MRI)
- Left ventricular systolic function (echocardiography or cardiac MRI)
- Hospitalization for heart failure
- Cardiac death

Table 7 Recommendations for designing cardioprotection studies in CABG patients

Patient selection

- Select patients undergoing on-pump cardiac surgery
- Select patients undergoing either CABG or valve surgery
- Select either stable or unstable patients

Peri-operative factors

- Standardize the anaesthetic regimen and concomitant medication where possible
- Select one myocardial preservation strategy only (blood cardioplegia, crystalloid cardioplegia, or cross-clamp fibrillation)

The therapeutic intervention

- Select a study intervention which has shown conclusive cardioprotection in pre-clinical studies
- Administer the study intervention either:
  (1) prior to aortic clamping (pre-ischaemia)
  (2) in the cardioplegic solution (after the onset of ischaemia)
  (3) at the time of aortic declamping (at reperfusion)

Choose cardioprotection-related study endpoints

- Incidence of type 5 CABG-related MI (see text for definition)
- Peri-operative myocardial injury or infarction (72 h AUC cardiac enzymes)
- Inotrope requirements
- Left ventricular systolic function (echocardiography)
- Hospitalization for heart failure
- Cardiac death

Supplementary material

Supplementary material is available at *Cardiovascular Research* online.

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References

1. Ludman AJ, Yellon DM, Hausenloy DJ. Cardiac preconditioning for ischaemia: lost in translation. *Dis Model Mech* 2010;**3**:35–38.
2. Hausenloy DJ, Baxter G, Bell R, Botker HE, Davidson SM, Downey J *et al*. Translating novel strategies for cardioprotection: the Hatter Workshop Recommendations. *Basic Res Cardiol* 2010;**105**:677–686.
3. Schwartz LL, Kloner RA, Arai AE, Baines CP, Bolli R, Braunwald E *et al*. New horizons in cardioprotection: recommendations from the 2010 national heart, lung, and blood institute workshop. *Circulation* 2011;**124**:1172–1179.
4. Heusch G. Cardioprotection-chances and challenges of its translation to the clinic. *Lancet* 2013;**381**:166–175.
5. Murry CE, Jennings RB, Reimer KA. Preconditioning with ischemia: a delay of lethal cell injury in ischemic myocardium. *Circulation* 1986;**74**:1124–1136.
6. Zhao ZQ, Corvera JS, Halkos ME, Kerendi F, Wang NP, Guyton RA *et al*. Inhibition of myocardial injury by ischemic postconditioning during reperfusion: comparison with ischemic preconditioning. *Am J Physiol Heart Circ Physiol* 2003;**285**:H579–H588.
7. Ovize M, Baxter GF, Di Lisa F, Ferdinandy P, Garcia-Dorado D, Hausenloy DJ *et al*. Postconditioning and protection from reperfusion injury: where do we stand? Position paper from the Working Group of Cellular Biology of the Heart of the European Society of Cardiology. *Cardiovasc Res* 2010;**87**:406–423.
8. Przyklenk K, Bauer B, Ovize M, Kloner RA, Whittaker P. Regional ischemic 'preconditioning' protects remote virgin myocardium from subsequent sustained coronary occlusion. *Circulation* 1993;**87**:893–899.
9. Hausenloy DJ, Yellon DM. Remote ischaemic preconditioning: underlying mechanisms and clinical application. *Cardiovasc Res* 2008;**79**:377–386.
10. Hausenloy DJ, Yellon DM. New directions for protecting the heart against ischaemia-reperfusion injury: targeting the Reperfusion Injury Salvage Kinase (RISK)-pathway. *Cardiovasc Res* 2004;**61**:448–460.
11. Hausenloy DJ, Yellon DM. Reperfusion injury salvage kinase signalling: taking a RISK for cardioprotection. *Heart Fail Rev* 2007;**12**:217–234.
12. Lecour S. Multiple protective pathways against reperfusion injury: a SAFE path without Aktion? *J Mol Cell Cardiol* 2009;**46**:607–609.
13. Lacerda L, Somers S, Opie LH, Lecour S. Ischaemic postconditioning protects against reperfusion injury via the SAFE pathway. *Cardiovasc Res* 2009;**84**:201–208.
14. Lecour S. Activation of the protective Survivor Activating Factor Enhancement (SAFE) pathway against reperfusion injury: does it go beyond the RISK pathway? *J Mol Cell Cardiol* 2009;**47**:32–40.
15. Hausenloy DJ, Lecour S, Yellon DM. Reperfusion injury salvage kinase and survivor activating factor enhancement prosurvival signaling pathways in ischemic postconditioning: two sides of the same coin. *Antioxid Redox Signal* 2011;**14**:893–907.
16. Skyschally A, Gres P, Hoffmann S, Haude M, Erbel R, Schulz R *et al*. Bidirectional role of tumor necrosis factor-alpha in coronary microembolization: progressive contractile dysfunction versus delayed protection against infarction. *Circ Res* 2007;**100**:140–146.
17. Boengler K, Hilfiker-Kleiner D, Drexler H, Heusch G, Schulz R. The myocardial JAK/STAT pathway: from protection to failure. *Pharmacol Ther* 2008;**120**:172–185.
18. Heusch G, Musiolik J, Kottenberg E, Peters J, Jakob H, Thielmann M. STAT5 activation and cardioprotection by remote ischemic preconditioning in humans: short communication. *Circ Res* 2012;**110**:111–115.
19. Boengler K, Buechert A, Heinen Y, Roeskes C, Hilfiker-Kleiner D, Heusch G *et al*. Cardioprotection by ischemic postconditioning is lost in aged and STAT3-deficient mice. *Circ Res* 2008;**102**:131–135.
20. Hausenloy DJ, Maddock HL, Baxter GF, Yellon DM. Inhibiting mitochondrial permeability transition pore opening: a new paradigm for myocardial preconditioning? *Cardiovasc Res* 2002;**55**:534–543.
21. Argaud L, Gateau-Roesch O, Raisky O, Loufouat J, Robert D, Ovize M. Postconditioning inhibits mitochondrial permeability transition. *Circulation* 2005;**111**:194–197.
22. Hausenloy DJ, Ong SB, Yellon DM. The mitochondrial permeability transition pore as a target for preconditioning and postconditioning. *Basic Res Cardiol* 2009;**104**:189–202.

23. Heusch G, Boengler K, Schulz R. Inhibition of mitochondrial permeability transition pore opening: the Holy Grail of cardioprotection. *Basic Res Cardiol* 2010;**105**: 151–154.
24. Heusch G, Boengler K, Schulz R. Cardioprotection: nitric oxide, protein kinases, and mitochondria. *Circulation* 2008;**118**:1915–1919.
25. Mandelzweig L, Battler A, Boyko V, Bueno H, Danchin N, Filippatos G *et al*. The second Euro Heart Survey on acute coronary syndromes: Characteristics, treatment, and outcome of patients with ACS in Europe and the Mediterranean Basin in 2004. *Eur Heart J* 2006;**27**:2285–2293.
26. Widimsky P, Wijns W, Fajadet J, de Belder M, Knot J, Aaberge L *et al*. Reperfusion therapy for ST elevation acute myocardial infarction in Europe: description of the current situation in 30 countries. *Eur Heart J* 2010;**31**:943–957.
27. Fox KA, Dabbous OH, Goldberg RJ, Pieper KS, Eagle KA, Van de Werf F *et al*. Prediction of risk of death and myocardial infarction in the six months after presentation with acute coronary syndrome: prospective multinational observational study (GRACE). *BMJ* 2006;**333**:1091.
28. Fox KA, Carruthers KF, Dunbar DR, Graham C, Manning JR, De RH *et al*. Underestimated and under-recognized: the late consequences of acute coronary syndrome (GRACE UK-Belgian Study). *Eur Heart J* 2010;**31**:2755–2764.
29. Mosterd A, Hoes AW. Clinical epidemiology of heart failure. *Heart* 2007;**93**: 1137–1146.
30. Ornato JP. The ST-segment-elevation myocardial infarction chain of survival. *Circulation* 2007;**116**:6–9.
31. Towae F, Juenger C, Mudra H, Glunz HG, Hauptmann E, Grube E *et al*. The development of door-to-angiography time in the last 14 years for patients with acute ST-elevation myocardial infarction treated with primary coronary intervention: determinants and outcome. Results from the MITRAplus and OPTAMI registry. *Acute Card Care* 2011;**13**:35–39.
32. Sorensen JT, Terkelsen CJ, Norgaard BL, Trautner S, Hansen TM, Botker HE *et al*. Urban and rural implementation of pre-hospital diagnosis and direct referral for primary percutaneous coronary intervention in patients with acute ST-elevation myocardial infarction. *Eur Heart J* 2011;**32**:430–436.
33. Yellon DM, Hausenloy DJ. Myocardial reperfusion injury. *N Engl J Med* 2007;**357**: 1121–1135.
34. Piper HM, Garcia-Dorado D, Ovize M. A fresh look at reperfusion injury. *Cardiovasc Res* 1998;**38**:291–300.
35. Krug A, Du Mesnil de R, Korb G. Blood supply of the myocardium after temporary coronary occlusion. *Circ Res* 1966;**19**:57–62.
36. Barrabes JA, Garcia-Dorado D, Mirabet M, Inserte J, Agullo L, Soriano B *et al*. Antagonism of selectin function attenuates microvascular platelet deposition and platelet-mediated myocardial injury after transient ischemia. *J Am Coll Cardiol* 2005; **45**:293–299.
37. Barrabes JA, Inserte J, Mirabet M, Quiroga A, Hernando V, Figueras J *et al*. Antagonism of P2Y12 or GPIIb/IIIa receptors reduces platelet-mediated myocardial injury after ischaemia and reperfusion in isolated rat hearts. *Thromb Haemost* 2010;**104**: 128–135.
38. Reffelmann T, Kloner RA. The no-reflow phenomenon: a basic mechanism of myocardial ischemia and reperfusion. *Basic Res Cardiol* 2006;**101**:359–372.
39. Heusch G, Kleinbongard P, Skyschally A, Levkau B, Schulz R, Erbel R. The coronary circulation in cardioprotection: more than just one confounder. *Cardiovasc Res* 2012; **94**:237–245.
40. Heusch G, Kleinbongard P, Bose D, Levkau B, Haude M, Schulz R *et al*. Coronary microembolization: from bedside to bench and back to bedside. *Circulation* 2009; **120**:1822–1836.
41. Oerlemans MI, Koudstaal S, Chamuleau SA, de Kleijn DP, Doevendans PA, Sluijter JP. Targeting cell death in the reperfused heart: Pharmacological approaches for cardioprotection. *Int J Cardiol*. Advance Access published March 27,2012, doi: 10.1016/j.ijcard.2012.03.055.
42. Brener SJ, Lytle BW, Schneider JP, Ellis SG, Topol EJ. Association between CK-MB elevation after percutaneous or surgical revascularization and three-year mortality. *J Am Coll Cardiol* 2002;**40**:1961–1967.
43. Kathiresan S, Servoss SJ, Newell JB, Trani D, MacGillivray TE, Lewandrowski K *et al*. Cardiac troponin T elevation after coronary artery bypass grafting is associated with increased one-year mortality. *Am J Cardiol* 2004;**94**:879–881.
44. Croal BL, Hillis GS, Gibson PH, Fazal MT, El Shafei H, Gibson G *et al*. Relationship between postoperative cardiac troponin I levels and outcome of cardiac surgery. *Circulation* 2006;**114**:1468–1475.
45. Thygesen K, Alpert JS, Jaffe AS, Simoons ML, Chaitman BR, White HD. Third universal definition of myocardial infarction. *Nat Rev Cardiol* 2012;**9**:620–633.
46. Venugopal V, Ludman A, Yellon DM, Hausenloy DJ. 'Conditioning' the heart during surgery. *Eur J Cardiothorac Surg* 2009;**35**:977–987.
47. Thielmann M, Massoudy P, Schmermund A, Neuhauser M, Marggraf G, Kamler M *et al*. Diagnostic discrimination between graft-related and non-graft-related perioperative myocardial infarction with cardiac troponin I after coronary artery bypass surgery. *Eur Heart J* 2005;**26**:2440–2447.
48. Hausenloy DJ, Boston-Griffiths E, Yellon DM. Cardioprotection during cardiac surgery. *Cardiovasc Res* 2012;**94**:253–265.
49. Ye S, Weng Y, Sun S, Chen W, Wu X, Li Z *et al*. Comparison of the durations of mild therapeutic hypothermia on outcome after cardiopulmonary resuscitation in the rat. *Circulation* 2012;**125**:123–129.
50. Cammarata G, Weil MH, Sun S, Tang W, Wang J, Huang L. Beta1-adrenergic blockade during cardiopulmonary resuscitation improves survival. *Crit Care Med* 2004;**32**: S440–S443.
51. Adams JA, Wu D, Bassuk J, Arias J, Lozano H, Kurlansky P *et al*. Nitric oxide synthase isoform inhibition before whole body ischemia reperfusion in pigs: vital or protective? *Resuscitation* 2007;**74**:516–525.
52. Tang W, Weil MH, Sun S, Pernat A, Mason E. K(ATP) channel activation reduces the severity of postresuscitation myocardial dysfunction. *Am J Physiol Heart Circ Physiol* 2000;**279**:H1609–H1615.
53. Radhakrishnan J, Kolarova JD, Ayoub IM, Gazmuri RJ. AVE4454B—a novel sodium-hydrogen exchanger isoform-1 inhibitor—compared less effective than cariporide for resuscitation from cardiac arrest. *Transl Res* 2011;**157**:71–80.
54. Huang CH, Hsu CY, Tsai MS, Wang TD, Chang WT, Chen WJ. Cardioprotective effects of erythropoietin on postresuscitation myocardial dysfunction in appropriate therapeutic windows. *Crit Care Med* 2008;**36**:S467–S473.
55. Gill RS, Manouchehri N, Liu JQ, Lee TF, Cho WJ, Thiesen A *et al*. Cyclosporine treatment improves cardiac function and systemic hemodynamics during resuscitation in a newborn piglet model of asphyxia: a dose-response study. *Crit Care Med* 2012;**40**:1237–1244.
56. Vassalli G, Milano G, Moccetti T. Role of mitogen-activated protein kinases in myocardial ischemia-reperfusion injury during heart transplantation. *J Transplant* 2012; **2012**:928954.
57. Inserte J, Barrabes JA, Hernando V, Garcia-Dorado D. Orphan targets for reperfusion injury. *Cardiovasc Res* 2009;**83**:169–178.
58. Mewton N, Elbaz M, Piot C, Ovize M. Infarct size reduction in patients with STEMI: why we can do it! *J Cardiovasc Pharmacol Ther* 2011;**16**:298–303.
59. Kloner RA, Schwartz LL. State of the science of cardioprotection: challenges and opportunities—proceedings of the 2010 NHLBI Workshop on Cardioprotection. *J Cardiovasc Pharmacol Ther* 2011;**16**:223–232.
60. Garcia-Dorado D, Ruiz-Meana M, Inserte J, Rodriguez-Sinovas A, Piper HM. Calcium-mediated cell death during myocardial reperfusion. *Cardiovasc Res* 2012; **94**:168–180.
61. Inserte J, Hernando V, Garcia-Dorado D. Contribution of calpains to myocardial ischaemia/reperfusion injury. *Cardiovasc Res* 2012;**96**:23–31.
62. Garcia-Dorado D, Agullo L, Sartorio CL, Ruiz-Meana M. Myocardial protection against reperfusion injury: the cGMP pathway. *Thromb Haemost* 2009;**101**:635–642.
63. Gotberg M, Olivecrona GK, Koul S, Carlsson M, Engblom H, Ugander M *et al*. A pilot study of rapid cooling by cold saline and endovascular cooling before reperfusion in patients with ST-elevation myocardial infarction. *Circ Cardiovasc Interv* 2010;**3**: 400–407.
64. O'Neill WW, Martin JL, Dixon SR, Bartorelli AL, Trabattoni D, Oemrawsingh PV *et al*. Acute Myocardial Infarction with Hyperoxemic Therapy (AMIHOT): a prospective, randomized trial of intracoronary hyperoxemic reperfusion after percutaneous coronary intervention. *J Am Coll Cardiol* 2007;**50**:397–405.
65. Staat P, Rioufol G, Piot C, Cottin Y, Cung TT, L'Huillier I *et al*. Postconditioning the human heart. *Circulation* 2005;**112**:2143–2148.
66. Botker HE, Kharbanda R, Schmidt MR, Bottcher M, Kaltoft AK, Terkelsen CJ *et al*. Remote ischaemic conditioning before hospital admission, as a complement to angioplasty, and effect on myocardial salvage in patients with acute myocardial infarction: a randomised trial. *Lancet* 2010;**375**:727–734.
67. Kitakaze M, Asakura M, Kim J, Shintani Y, Asanuma H, Hamasaki T *et al*. Human atrial natriuretic peptide and nicorandil as adjuncts to reperfusion treatment for acute myocardial infarction (J-WIND): two randomised trials. *Lancet* 2007;**370**:1483–1493.
68. Piot C, Croisille P, Staat P, Thibault H, Rioufol G, Mewton N *et al*. Effect of cyclosporine on reperfusion injury in acute myocardial infarction. *N Engl J Med* 2008;**359**: 473–481.
69. Lonborg J, Vejlstrup N, Kelbaek H, Botker HE, Kim WY, Mathiasen AB *et al*. Exenatide reduces reperfusion injury in patients with ST-segment elevation myocardial infarction. *Eur Heart J* 2012;**33**:1491–1499.
70. Ovize M. Cyclosporine and Prognosis in Acute Myocardial Infarction (MI) Patients (CIRCUS).www.clinicaltrials.gov, 2012.
71. Engstrom T. DANish Study of Optimal Acute Treatment of Patients With ST-elevation Myocardial Infarction (DANAMI-3) NCT01435408. www.clinicaltrials.gov, 2012.
72. Selker HP, Beshansky JR, Sheehan PR, Massaro JM, Griffith JL, D'Agostino RB *et al*. Out-of-hospital administration of intravenous glucose-insulin-potassium in patients with suspected acute coronary syndromes: the IMMEDIATE randomized controlled trial. *JAMA* 2012;**307**:1925–1933.
73. Sato H, Jordan JE, Zhao ZQ, Sarvotham SS, Vinten-Johansen J. Gradual reperfusion reduces infarct size and endothelial injury but augments neutrophil accumulation. *Ann Thorac Surg* 1997;**64**:1099–1107.
74. Bopassa JC, Michel P, Gateau-Roesch O, Ovize M, Ferrera R. Low-pressure reperfusion alters mitochondrial permeability transition. *Am J Physiol Heart Circ Physiol* 2005; **288**:H2750–H2755.

75. Musiolik J, van Caster P, Skyschally A, Boengler K, Gres P, Schulz R *et al*. Reduction of infarct size by gentle reperfusion without activation of reperfusion injury salvage kinases in pigs. *Cardiovasc Res* 2010;**85**:110–117.
76. Heusch G. Postconditioning: old wine in a new bottle? *J Am Coll Cardiol* 2004;**44**: 1111–1112.
77. Manintveld OC, Te Lintel HM, van den Bos EJ, Suurenbroek GM, Dekkers D, Verdouw PD *et al*. Cardiac effects of postconditioning depend critically on the duration of index ischemia. *Am J Physiol Heart Circ Physiol* 2007;**292**:H1551–1560.
78. Ruiz-Meana M, Inserte J, Fernandez-Sanz C, Hernando V, Miro-Casas E, Barba I *et al*. The role of mitochondrial permeability transition in reperfusion-induced cardiomyocyte death depends on the duration of ischemia. *Basic Res Cardiol* 2011;**106**: 1259–1268.
79. Reimer KA, Lowe JE, Rasmussen MM, Jennings RB. The wavefront phenomenon of ischemic cell death. 1. Myocardial infarct size vs duration of coronary occlusion in dogs. *Circulation* 1977;**56**:786–794.
80. Ortiz-Perez JT, Meyers SN, Lee DC, Kansal P, Klocke FJ, Holly TA *et al*. Angiographic estimates of myocardium at risk during acute myocardial infarction: validation study using cardiac magnetic resonance imaging. *Eur Heart J* 2007;**28**:1750–1758.
81. Botker HE, Kaltoft AK, Pedersen SF, Kim WY. Measuring myocardial salvage. *Cardiovasc Res* 2012;**94**:266–275.
82. Mewton N, Rapacchi S, Augeul L, Ferrera R, Loufouat J, Boussel L *et al*. Determination of the myocardial area at risk with pre- versus post-reperfusion imaging techniques in the pig model. *Basic Res Cardiol* 2011;**106**:1247–1257.
83. Thuny F, Lairez O, Roubille F, Mewton N, Rioufol G, Sportouch C *et al*. Post-conditioning reduces infarct size and edema in patients with ST-segment elevation myocardial infarction. *J Am Coll Cardiol* 2012;**59**:2175–2181.
84. Wince WB, Kim RJ. Molecular imaging: T2-weighted CMR of the area at risk—a risky business? *Nat Rev Cardiol* 2010;**7**:547–549.
85. Thibault H, Piot C, Staat P, Bontemps L, Sportouch C, Rioufol G *et al*. Long-term benefit of postconditioning. *Circulation* 2008;**117**:1037–1044.
86. Miura T, Miki T. Limitation of myocardial infarct size in the clinical setting: current status and challenges in translating animal experiments into clinical therapy. *Basic Res Cardiol* 2008;**103**:501–513.
87. Reimer KA, Jennings RB. The 'wavefront phenomenon' of myocardial ischemic cell death. II. Transmural progression of necrosis within the framework of ischemic bed size (myocardium at risk) and collateral flow. *Lab Invest* 1979;**40**:633–644.
88. Ferdinandy P, Schulz R, Baxter GF. Interaction of cardiovascular risk factors with myocardial ischemia/reperfusion injury, preconditioning, and postconditioning. *Pharmacol Rev* 2007;**59**:418–458.
89. Ferdinandy P, Szilvassy Z, Baxter GF. Adaptation to myocardial stress in disease states: is preconditioning a healthy heart phenomenon? *Trends Pharmacol Sci* 1998; **19**:223–229.
90. Loubani M, Ghosh S, Galinanes M. The aging human myocardium: tolerance to ischemia and responsiveness to ischemic preconditioning. *J Thorac Cardiovasc Surg* 2003; **126**:143–147.
91. Hassouna A, Loubani M, Matata BM, Fowler A, Standen NB, Galinanes M. Mitochondrial dysfunction as the cause of the failure to precondition the diabetic human myocardium. *Cardiovasc Res* 2006;**69**:450–458.
92. Sivaraman V, Hausenloy DJ, Wynne AM, Yellon DM. Preconditioning the diabetic human myocardium. *J Cell Mol Med* 2010;**14**:1740–1746.
93. Roubille F, Lairez O, Mewton N, Rioufol G, Ranc S, Sanchez I *et al*. Cardioprotection by clopidogrel in acute ST-elevated myocardial infarction patients: a retrospective analysis. *Basic Res Cardiol* 2012;**107**:275.
94. Bein B. Clinical application of the cardioprotective effects of volatile anaesthetics: PRO—get an extra benefit from a proven anaesthetic free of charge. *Eur J Anaesthesiol* 2011;**28**:620–622.
95. Przyklenk K. Efficacy of cardioprotective 'conditioning' strategies in aging and diabetic cohorts: the co-morbidity conundrum. *Drugs Aging* 2011;**28**:331–343.
96. Loubani M, Fowler A, Standen NB, Galinanes M. The effect of gliclazide and glibenclamide on preconditioning of the human myocardium. *Eur J Pharmacol* 2005;**515**: 142–149.
97. Carr CS, Yellon DM. Ischaemic preconditioning may abolish the protection afforded by ATP-sensitive potassium channel openers in isolated human atrial muscle. *Basic Res Cardiol* 1997;**92**:252–260.
98. Yang X-M, Liu Y, Cui L, Yang X, Liu Y, Tandon N *et al*. Platelet P2Y12 blockers confer direct postconditioning-like protection in reperfused rabbit hearts. *J Cardiovasc Pharmacol Ther*. Advance Access published December 10, 2010, doi: 10.1177/1074248412467692.
99. Yang X-M, Liu Y, Cui L, Yang X, Liu Y, Tandon N,*et al*. Two classes of anti-platelet drugs reduce anatomical infarct size in monkey hearts. *Cardiovasc Drugs Ther*. Advance Access published January 15, 2013, doi: 10.1007/s10557-012-6436-7.
100. Tarantini G, Favaretto E, Marra MP, Frigo AC, Napodano M, Cacciavillani L *et al*. Postconditioning during coronary angioplasty in acute myocardial infarction: the POST-AMI trial. *Int J Cardiol* 2012;**162**:33–38.
101. Freixa X, Bellera N, Ortiz-Perez JT, Jimenez M, Pare C, Bosch X *et al*. Ischaemic postconditioning revisited: lack of effects on infarct size following primary percutaneous coronary intervention. *Eur Heart J* 2012;**33**:103–112.
102. Hausenloy DJ, Yellon DM. 'Conditional Conditioning' in cardiac bypass surgery. *Basic Res Cardiol* 2012;**107**:1–6.
103. Hausenloy DJ, Candilio L, Laing C, Kunst G, Pepper J, Kolvekar S *et al*. Effect of remote ischemic preconditioning on clinical outcomes in patients undergoing coronary artery bypass graft surgery (ERICCA): rationale and study design of a multicentre randomized double-blinded controlled clinical trial. *Clin Res Cardiol* 2012; **101**:339–348.
104. Meybohm P, Zacharowski K, Cremer J, Roesner J, Kletzin F, Schaelte G *et al*. Remote ischaemic preconditioning for heart surgery. The study design for a multi-center randomized double-blinded controlled clinical trial—the RIPHeart-Study. *Eur Heart J* 2012;**33**:1423–1426.
105. Heusch G. Nitroglycerin and delayed preconditioning in humans: yet another new mechanism for an old drug? *Circulation* 2001;**103**:2876–2878.
106. THE EMIP-FR GROUP. Effect of 48-h intravenous trimetazidine on short- and long-term outcomes of patients with acute myocardial infarction, with and without thrombolytic therapy: a double-blind, placebo-controlled, randomized trial. The EMIP-FR Group. European Myocardial Infarction Project—Free Radicals. *Eur Heart J* 2000;**21**:1537–1546.
107. Early administration of intravenous magnesium to high-risk patients with acute myocardial infarction in the Magnesium in Coronaries (MAGIC) Trial: a randomised controlled trial. *Lancet* 2002;**360**:1189–1196.
108. Mehta SR, Yusuf S, Diaz R, Zhu J, Pais P, Xavier D *et al*. Effect of glucose-insulin-potassium infusion on mortality in patients with acute ST-segment elevation myocardial infarction: the CREATE-ECLA randomized controlled trial. *JAMA* 2005;**293**:437–446.
109. Zeymer U, Suryapranata H, Monassier JP, Opolski G, Davies J, Rasmanis G *et al*. The Na(+)/H(+) exchange inhibitor eniporide as an adjunct to early reperfusion therapy for acute myocardial infarction. Results of the evaluation of the safety and cardioprotective effects of eniporide in acute myocardial infarction (ESCAMI) trial. *J Am Coll Cardiol* 2001;**38**:1644–1650.
110. Armstrong PW, Granger CB, Adams PX, Hamm C, Holmes D Jr, O'Neill WW *et al*. Pexelizumab for acute ST-elevation myocardial infarction in patients undergoing primary percutaneous coronary intervention: a randomized controlled trial. *JAMA* 2007;**297**:43–51.
111. Atar D, Petzelbauer P, Schwitter J, Huber K, Rensing B, Kasprzak JD *et al*. Effect of intravenous FX06 as an adjunct to primary percutaneous coronary intervention for acute ST-segment elevation myocardial infarction results of the F.I.R.E. (Efficacy of FX06 in the Prevention of Myocardial Reperfusion Injury) trial. *J Am Coll Cardiol* 2009;**53**:720–729.
112. Voors AA, Belonje AM, Zijlstra F, Hillege HL, Anker SD, Slart RH *et al*. A single dose of erythropoietin in ST-elevation myocardial infarction. *Eur Heart J* 2010;**31**: 2593–2600.
113. Ott I, Schulz S, Mehilli J, Fichtner S, Hadamitzky M, Hoppe K *et al*. Erythropoietin in patients with acute ST-segment elevation myocardial infarction undergoing primary percutaneous coronary intervention: a randomized, double-blind trial. *Circ Cardiovasc Interv* 2010;**3**:408–413.
114. Ludman AJ, Yellon DM, Hasleton J, Ariti C, Babu GG, Boston-Griffiths E *et al*. Effect of erythropoietin as an adjunct to primary percutaneous coronary intervention: a randomised controlled clinical trial. *Heart* 2011;**97**:1560–1565.
115. Hahn JY, Kim HJ, Choi YJ, Jo SH, Kim HJ, Lee S *et al*. Effects of atorvastatin pretreatment on infarct size in patients with ST-segment elevation myocardial infarction undergoing primary percutaneous coronary intervention. *Am Heart J* 2011;**162**: 1026–1033.
116. Post S, Post MC, van den Branden BJ, Eefting FD, Goumans MJ, Stella PR *et al*. Early statin treatment prior to primary PCI for acute myocardial infarction: REPERATOR, a randomized placebo-controlled pilot trial. *Catheter Cardiovasc Interv* 2012;**80**: 756–765.
117. Chan W, Taylor AJ, Ellims AH, Lefkovits L, Wong C, Kingwell BA *et al*. Effect of iron chelation on myocardial infarct size and oxidative stress in ST-elevation-myocardial infarction. *Circ Cardiovasc Interv* 2012;**5**:270–278.
118. Lonborg J, Kelbaek H, Vejlstrup N, Jorgensen E, Helqvist S, Saunamaki K *et al*. Cardioprotective effects of ischemic postconditioning in patients treated with primary percutaneous coronary intervention, evaluated by magnetic resonance. *Circ Cardiovasc Interv* 2010;**3**:34–41.
119. Sorensson P, Saleh N, Bouvier F, Bohm F, Settergren M, Caidahl K *et al*. Effect of postconditioning on infarct size in patients with ST elevation myocardial infarction. *Heart* 2010;**96**:1710–1715.
120. Mewton N, Croisille P, Gahide G, Rioufol G, Bonnefoy E, Sanchez I *et al*. Effect of cyclosporine on left ventricular remodeling after reperfused myocardial infarction. *J Am Coll Cardiol* 2010;**55**:1200–1205.
121. Stone GW, Martin JL, de Boer MJ, Margheri M, Bramucci E, Blankenship JC *et al*. Effect of supersaturated oxygen delivery on infarct size after percutaneous coronary intervention in acute myocardial infarction. *Circ Cardiovasc Interv* 2009;**2**:366–375.
122. Munk K, Andersen NH, Schmidt MR, Nielsen SS, Terkelsen CJ, Sloth E *et al*. Remote ischemic conditioning in patients with myocardial infarction treated with primary angioplasty: impact on left ventricular function assessed by comprehensive echocardiography and gated single-photon emission CT. *Circ Cardiovasc Imaging* 2010;**3**: 656–662.

123. Rentoukas I, Giannopoulos G, Kaoukis A, Kossyvakis C, Raisakis K, Driva M *et al*. Cardioprotective role of remote ischemic periconditioning in primary percutaneous coronary intervention: enhancement by opioid action. *JACC Cardiovasc Interv* 2010;**3**: 49–55.
124. Lonborg J, Kelbaek H, Vejlstrup N, Botker HE, Kim WY, Holmvang L *et al*. Exenatide reduces final infarct size in patients with ST-segment-elevation myocardial infarction and short-duration of ischemia. *Circ Cardiovasc Interv* 2012;**5**:288–295.
125. Boyce SW, Bartels C, Bolli R, Chaitman B, Chen JC, Chi E *et al*. Impact of sodium-hydrogen exchange inhibition by cariporide on death or myocardial infarction in high-risk CABG surgery patients: results of the CABG surgery cohort of the GUARDIAN study. *J Thorac Cardiovasc Surg* 2003;**126**:420–427.
126. Mentzer RM Jr, Bartels C, Bolli R, Boyce S, Buckberg GD, Chaitman B *et al*. Sodium-hydrogen exchange inhibition by cariporide to reduce the risk of ischemic cardiac events in patients undergoing coronary artery bypass grafting: results of the EXPED-ITION study. *Ann Thorac Surg* 2008;**85**:1261–1270.
127. Mangano DT, Miao Y, Tudor IC, Dietzel C. Post-reperfusion myocardial infarction: long-term survival improvement using adenosine regulation with acadesine. *J Am Coll Cardiol* 2006;**48**:206–214.
128. Newman MF, Ferguson TB, White JA, Ambrosio G, Koglin J, Nussmeier NA *et al*. Effect of adenosine-regulating agent acadesine on morbidity and mortality associated with coronary artery bypass grafting: the RED-CABG randomized controlled trial. *JAMA* 2012;**308**:157–164.
129. Verrier ED, Shernan SK, Taylor KM, Van De WF, Newman MF, Chen JC *et al*. Terminal complement blockade with pexelizumab during coronary artery bypass graft surgery requiring cardiopulmonary bypass: a randomized trial. *JAMA* 2004;**291**: 2319–2327.
130. Smith PK, Shernan SK, Chen JC, Carrier M, Verrier ED, Adams PX *et al*. Effects of C5 complement inhibitor pexelizumab on outcome in high-risk coronary artery bypass grafting: combined results from the PRIMO-CABG I and II trials. *J Thorac Cardiovasc Surg* 2011;**142**:89–98.
131. Cheung MM, Kharbanda RK, Konstantinov IE, Shimizu M, Frndova H, Li J *et al*. Randomized controlled trial of the effects of remote ischemic preconditioning on children undergoing cardiac surgery: first clinical application in humans. *J Am Coll Cardiol* 2006;**47**:2277–2282.
132. Hausenloy DJ, Mwamure PK, Venugopal V, Harris J, Barnard M, Grundy E *et al*. Effect of remote ischaemic preconditioning on myocardial injury in patients undergoing coronary artery bypass graft surgery: a randomised controlled trial. *Lancet* 2007; **370**:575–579.
133. Ali ZA, Callaghan CJ, Lim E, Ali AA, Nouraei SA, Akthar AM *et al*. Remote ischemic preconditioning reduces myocardial and renal injury after elective abdominal aortic aneurysm repair: a randomized controlled trial. *Circulation* 2007;**116**:I98–105.
134. Venugopal V, Hausenloy DJ, Ludman A, Di Salvo C, Kolvekar S, Yap J *et al*. Remote ischaemic preconditioning reduces myocardial injury in patients undergoing cardiac surgery with cold-blood cardioplegia: a randomised controlled trial. *Heart* 2009;**95**: 1567–1571.
135. Wagner R, Piler P, Bedanova H, Adamek P, Grodecka L, Freiberger T. Myocardial injury is decreased by late remote ischaemic preconditioning and aggravated by tramadol in patients undergoing cardiac surgery: a randomised controlled trial. *Interact Cardiovasc Thorac Surg* 2010;**11**:758–762.
136. Thielmann M, Kottenberg E, Boengler K, Raffelsieper C, Neuhaeuser M, Peters J *et al*. Remote ischemic preconditioning reduces myocardial injury after coronary artery bypass surgery with crystalloid cardioplegic arrest. *Basic Res Cardiol* 2010; **105**:657–664.
137. Li L, Luo W, Huang L, Zhang W, Gao Y, Jiang H *et al*. Remote perconditioning reduces myocardial injury in adult valve replacement: a randomized controlled trial. *J Surg Res* 2010;**164**:e21–e26.
138. Choi YS, Shim JK, Kim JC, Kang KS, Seo YH, Ahn KR *et al*. Effect of remote ischemic preconditioning on renal dysfunction after complex valvular heart surgery: a randomized controlled trial. *J Thorac Cardiovasc Surg* 2011;**142**:148–154.
139. Wu Q, Gui P, Wu J, Ding D, Purusram G, Dong N *et al*. Effect of limb ischemic preconditioning on myocardial injury in patients undergoing mitral valve replacement surgery. A randomized controlled trial. *Circ J* 2011;**75**:1885–1889.
140. Kottenberg E, Thielmann M, Bergmann L, Heine T, Jakob H, Heusch G *et al*. Protection by remote ischemic preconditioning during coronary artery bypass graft surgery with isoflurane but not propofol: a clinical trial. *Acta Anaesthesiol Scand* 2012;**56**: 30–38.
141. Xie JJ, Liao XL, Chen WG, Huang DD, Chang FJ, Chen W *et al*. Remote ischaemic preconditioning reduces myocardial injury in patients undergoing heart valve surgery: randomised controlled trial. *Heart* 2012;**98**:384–388.
142. Rahman IA, Mascaro JG, Steeds RP, Frenneaux MP, Nightingale P, Gosling P *et al*. Remote ischemic preconditioning in human coronary artery bypass surgery: from promise to disappointment? *Circulation* 2010;**122**:S53–S59.
143. Karuppasamy P, Chaubey S, Dew T, Musto R, Sherwood R, Desai J *et al*. Remote intermittent ischemia before coronary artery bypass graft surgery: a strategy to reduce injury and inflammation? *Basic Res Cardiol* 2011;**106**:511–519.
144. Lucchinetti E, Bestmann L, Feng J, Freidank H, Clanachan AS, Finegan BA *et al*. Remote ischemic preconditioning applied during isoflurane inhalation provides no benefit to the myocardium of patients undergoing on-pump coronary artery bypass graft surgery: lack of synergy or evidence of antagonism in cardioprotection? *Anesthesiology* 2012;**116**:296–310.
145. Young PJ, Dalley P, Garden A, Horrocks C, La FA, Mahon B *et al*. A pilot study investigating the effects of remote ischemic preconditioning in high-risk cardiac surgery using a randomised controlled double-blind protocol. *Basic Res Cardiol* 2012;**107**:1–10.
146. Lomivorotov VV, Shmyrev VA, Nepomnyaschih VA, Ponomarev DN, Knyazkova LG, Lomivorotov VN *et al*. Remote ischaemic preconditioning does not protect the heart in patients undergoing coronary artery bypass grafting. *Interact Cardiovasc Thorac Surg* 2012;**15**:18–22.
147. Luo W, Li B, Lin G, Huang R. Postconditioning in cardiac surgery for tetralogy of Fallot. *J Thorac Cardiovasc Surg* 2007;**133**:1373–1374.
148. Luo W, Li B, Chen R, Huang R, Lin G. Effect of ischemic postconditioning in adult valve replacement. *Eur J Cardiothorac Surg* 2008;**33**:203–208.
149. Li B, Chen R, Huang R, Luo W. Clinical benefit of cardiac ischemic postconditioning in corrections of tetralogy of Fallot. *Interact Cardiovasc Thorac Surg* 2009;**8**:17–21.
150. Ranasinghe AM, Quinn DW, Pagano D, Edwards N, Faroqui M, Graham TR *et al*. Glucose-insulin-potassium and tri-iodothyronine individually improve hemodynamic performance and are associated with reduced troponin I release after on-pump coronary artery bypass grafting. *Circulation* 2006;**114**:I245–I250.
151. Symons JA, Myles PS. Myocardial protection with volatile anaesthetic agents during coronary artery bypass surgery: a meta-analysis. *Br J Anaesth* 2006;**97**:127–136.
152. Yu CH, Beattie WS. The effects of volatile anesthetics on cardiac ischemic complications and mortality in CABG: a meta-analysis. *Can J Anaesth* 2006;**53**:906–918.
153. Landoni G, Biondi-Zoccai GG, Zangrillo A, Bignami E, D'Avolio S, Marchetti C *et al*. Desflurane and sevoflurane in cardiac surgery: a meta-analysis of randomized clinical trials. *J Cardiothorac Vasc Anesth* 2007;**21**:502–511.