Ischaemic conditioning and reperfusion injury

Derek J. Hausenloy[1,2] and Derek M. Yellon[2]

[1]Cardiovascular and Metabolic Disorders Program, Duke-National University of Singapore, Singapore 169857, Singapore; National Heart Research Institute Singapore, National Heart Centre Singapore, 5 Hospital Drive. Singapore 169609

[2]The Hatter Cardiovascular Institute, University College London, 67 Chenies Mews, London WC1E 6HX, UK.

Correspondence to D.M.Y.

d.yellon@ucl.ac.uk

Abstract | 2016 will be the 30-year anniversary of the discovery of 'ischaemic preconditioning'. This endogenous phenomenon can paradoxically protect the heart from acute myocardial infarction by subjecting it to one or more brief cycles of ischaemia and reperfusion. After complete reperfusion, this method is the most powerful intervention known for reducing infarct size. The concept of ischaemic preconditioning has evolved into 'ischaemic conditioning', a term that encompasses a number of related endogenous cardioprotective strategies, applied either directly to the heart (ischaemic preconditioning or postconditioning) or from afar, for example a limb (remote ischaemic preconditioning, perconditioning, or postconditioning). Investigations of signalling pathways underlying ischaemic conditioning have identified a number of therapeutic targets for pharmacological manipulation. Over the past 3 decades, a number of ischaemic and pharmacological cardioprotection strategies, discovered in experimental

studies, have been examined in the clinical setting of acute myocardial infarction and CABG surgery. The results have been disappointing, and no effective cardioprotective therapy is currently used in clinical practice. Several large, multicentre, randomized, controlled clinical trials on cardioprotection have highlighted the challenges of translating ischaemic conditioning and pharmacological cardioprotection strategies into patient benefit. However, in the past a number of cardioprotective therapies have shown promising results in reducing infarct size and improving clinical outcomes in patients with ischaemic heart disease.

Ischaemic heart disease (IHD) is the leading cause of death and disability worldwide. ST-segment elevation myocardial infarction (STEMI) is a major emergency manifestation of IHD, usually precipitated by acute thrombotic occlusion of a main coronary artery at the site of a ruptured atherosclerotic plaque. The treatment of choice for reducing the size of a myocardial infarct (MI), preserving left ventricular (LV) systolic function, and preventing the onset of heart failure in patients with STEMI is reperfusion, using primary percutaneous coronary intervention (PPCI). However, despite timely PPCI, mortality and morbidity in patients with STEMI remain substantial, with death reported in 7% and heart failure in 22% of patients at 1 year after the event[1]. Although mortality of patients with STEMI has declined over the past 10-15 years owing to improvements in secondary preventative therapy, the number of patients who develop heart failure has increased. The size of an MI is a major determinant of LV systolic function and the propensity for developing heart failure; therefore novel therapies that

reduce infarct size and can be administered as adjuncts to PPCI are needed to improve patient survival and prevent the onset of heart failure.

In patients with IHD and severe multivessel coronary disease, the heart is more commonly revascularized using CABG surgery. This operation can be complicated by perioperative myocardial injury (PMI), which can result from acute global myocardial ischaemia–reperfusion injury (IRI) when going onto and coming off cardiopulmonary bypass[2, 3], and can lead to LV systolic impairment, the onset of heart failure, and risk of death after surgery. Owing to the ageing population and the growing prevalence of comorbidities (such as diabetes mellitus, obesity, hypertension, and valve disease), patients undergoing CABG surgery are at a higher risk of PMI than ever before. The extent of PMI is a critical determinant of clinical outcomes after surgery[4-6]. Novel therapies are, therefore, required for this patient group to reduce the magnitude of PMI, preserve LV systolic function, and prevent the onset of heart failure. Notably, myocardial injury and cardiomyocyte death after CABG surgery is caused by acute IRI similar to revascularization after STEMI; however, factors such as direct handling of the heart, coronary microembolization, and inflammation also contribute and might influence effectiveness of therapies. For both patient groups, 'ischaemic conditioning' provides an endogenous strategy that can protect the heart from the detrimental effects of acute IRI, and which has the potential to improve clinical outcomes in patients with IHD. Ischaemic conditioning is the term given to a number of related endogenous cardioprotective strategies, all based on rendering the heart tolerant to acute IRI by conditioning it with one or more brief cycles of ischaemia and reperfusion (FIG. 1). In this Review, we provide an overview of the various types of ischaemic conditioning and pharmacological

cardioprotection. The challenges of translating these methods into the clinical setting are highlighted, and future therapies that have the potential to improve clinical outcomes in patients with IHD are discussed.

[H1] Myocardial reperfusion injury

In 1985, Braunwald & Kloner wrote that “myocardial reperfusion may be viewed as a double-edged sword”[7]. Although strategies are in place to minimize acute myocardial ischaemic injury for patients presenting with an acute STEMI or in patients undergoing cardiac surgery, ‘myocardial reperfusion injury’ — the myocardial injury and cardiomyocyte death that paradoxically occurs with the acute reperfusion of ischaemic myocardium — remains a neglected therapeutic target in both these patient groups[8-11]. Therefore, although myocardial reperfusion is essential to salvage viable myocardium, it comes at a price.

In 1960, Jennings *et al*. first suggested that reperfusion might hasten the necrotic process of cardiomyocytes irreversibly injured during ischaemia.[12] More contentious was the notion that reperfusion could induce the death of cardiomyocytes[7] which had only been reversibly injured during ischemia. However, the experimental and clinical evidence for myocardial reperfusion injury has been convincingly provided by the observation that a therapeutic intervention applied solely at the onset of reperfusion can reduce MI size[9, 11].

Myocardial reperfusion injury can manifest in four different forms. Reperfusion-induced arrhythmias can be induced in patients with STEMI on acutely reperfusing ischaemic myocardium through thrombolysis or PPCI and comprise idioventricular

rhythm and ventricular arrhythmias. The majority of these arrhythmias are self-terminating or are easily managed. Myocardial stunning is a reversible contractile dysfunction that also occurs on acutely reperfusing ischaemic myocardium, and is believed to result from oxidative stress and intracellular calcium overload in the cardiomyocyte. Myocardial stunning is usually self-terminating, with myocardial function recovering within days or weeks. Microvascular obstruction (MVO), the third form of myocardial reperfusion injury, was originally defined by Krug *et al.* in 1966 as the "inability to reperfuse a previously ischaemic region"[13], and its histological features were characterized by Kloner *et al.* in 1974 in the canine heart[14]. The aetiology of MVO is multifactorial and has been attributed to a number of events, including capillary damage with impaired vasodilatation, external capillary compression by endothelial cells, cardiomyocyte swelling, microembolization of friable material released from the atherosclerotic plaque, platelet microthrombi, and neutrophil plugging[15, 16]. Among patients with STEMI, MVO has been reported to occur in up to 60% of patients with post-PPCI normal coronary flow (TIMI 3) within the infarct-related artery[16, 17]. The presence of MVO is associated with adverse LV remodelling, and poor clinical outcomes post-PPCI. Lethal myocardial reperfusion injury is the main cause of reperfusion-induced death of cardiomyocytes that have been reversibly injured, and can contribute to up to 50% of the final MI size. Cytosolic and mitochondrial calcium overload, oxidative stress, and rapid restoration of intracellular pH, which result in the opening of the mitochondrial permeability transition pore (MPTP) and irreversible cardiomyocyte hypercontracture[18], have been shown to trigger lethal myocardial reperfusion injury. However, a number of other factors contribute, including osmotic

overload, gap-junction changes, and inflammatory signalling. Crucially, no effective therapy exists for reducing lethal myocardial reperfusion injury in patients with STEMI who have undergone revascularization procedures or after cardiac surgery

[H1] Ischaemic preconditioning

While investigating the cumulative effects of short periods of ischaemia on myocardial adenine nucleotides, lactate, and MI size, Murry, Reimer, and Jennings made the intriguing discovery that 4–5 min cycles of alternating occlusion and reflow of the left anterior descending (LAD) coronary artery, applied immediately before 90 min of occlusion and 3 days of reperfusion, resulted in a 75% reduction in MI size in the canine heart.[19, 20] This seminal observation, termed ischaemic preconditioning (IPC), has been replicated in all species tested, including humans, and can be readily applied to other organs and tissues[21-23]. After reperfusion, IPC remains the most powerful intervention for reducing MI size in ischaemic hearts. Over the past 3 decades, almost 9,000 papers have been published on this topic.

[H2] Mechanisms of action

The IPC stimulus has been shown to induce two distinct windows of cardioprotection. The first window occurs immediately after the IPC stimulus and lasts 2–3 h (termed ‘classical IPC’ or ‘acute IPC’), after which the effect wanes and disappears. The second window follows 12–24 h later, and lasts 48–72 h (termed ‘delayed IPC’ or ‘second window of protection’ [SWOP])[24, 25]. Despite intensive investigation, the actual mechanisms that mediate this cardioprotective effect remain incompletely understood,

although a large number of signalling pathways underlying IPC have been identified. Only a very simplified overview can be provided in this Review (more-comprehensive reviews of the subject have been published previously[21, 22, 26]).

In brief, the IPC stimulus, made up of cycles of brief ischaemia and reperfusion, initiates production of a number of autacoids (such as acetylcholine, adenosine, bradykinin, endothelin, and opioids) by cardiomyocytes. These autacoids bind to their respective receptors on the plasma membrane of cardiomyocytes to stimulate a number of signalling pathways that convey a cardioprotective signal to the mitochondria. In the mitochondria, signalling reactive oxygen species (ROS) are generated and activate protein kinases such as Akt, Erk1/2, protein kinase C, and tyrosine kinase, which provide the 'memory'. This process allows the cardioprotective effect to last up to 2–3 h (in classical IPC). In delayed IPC, these protein kinases activate transcription factors (such as AP-1, hypoxic-inducible factor 1α, nuclear factor κB, nuclear factor erythroid 2-related factor 2 [also known as Nrf2], and signal transducer and activator of transcription [STAT] 1/3), which facilitate the synthesis of 'distal mediators' (such as prostaglandin G/H synthase [also known as COX-2], heat shock proteins (such as HSP72), and inducible nitric oxide synthase), which in turn induce the cardioprotective effect 12–24 h after the IPC stimulus[27]. In the prevention of myocardial reperfusion injury, IPC has been shown to recruit prosurvival signalling pathways at the onset of reperfusion, including the Reperfusion Injury Salvage Kinase (RISK) pathway (comprising Akt and Erk1/2)[28] and the Survivor Activator Factor Enhancement (SAFE) pathway (comprising TNF and JAK–STAT3)[29]. The final processes of cardioprotection in classical and delayed IPC remain unclear, although some investigators have

hypothesized that preservation of mitochondrial function with less calcium overload, attenuated ROS production, and MPTP inhibition might contribute to the protective effect[21, 22, 30] (FIG. 2). Functional genomics of myocardial tissue has the potential to provide further insights into the mechanisms underlying IPC and other endogenous cardioprotective strategies[31].

[H2] Clinical application

The phenomenon of IPC can be observed in a number of clinical scenarios in which the heart protects itself with brief episodes of ischaemia. 'Warm-up angina' refers to the phenomenon of increased exercise tolerance following an episode of angina after a period of rest[32]. 'Pre-infarct angina' is defined as the cardioprotective effect of antecedent angina before an acute MI resulting in smaller infarct size and improved clinical outcomes[33].

The first clinical study conducted to test external application of an IPC stimulus in patients undergoing CABG surgery was undertaken in 1993 by our group[34]. We found that intermittent clamping and declamping of the aorta preserved myocardial ATP levels in a manner similar to that seen by Murry and colleagues, as described in their seminal paper on preconditioning[19]. Since 1993, a number of studies have confirmed the cardioprotective effect of IPC by reducing the extent of PMI (as measured by serum cardiac enzymes) in patients undergoing CABG surgery. A meta-analysis of 22 trials, which included data for a total of 933 patients, found that application of IPC reduced ventricular arrhythmias, decreased inotrope requirements, and shortened the length of stay in an intensive care unit compared with control[35] . Despite these potential beneficial

effects, the need to intervene on the heart directly and the inherent risk of thromboembolization arising from clamping an atherosclerotic aorta have prevented IPC from being adopted in this clinical setting.

[H1] Ischaemic postconditioning

The major disadvantage of IPC as a cardioprotective strategy is the requirement to intervene before occurrence of the ischaemic event, which is not possible in the case of an acute MI. In 2003, Zhao *et al.* made the exciting discovery that the heart could be protected against acute MI by interrupting myocardial reperfusion with several short-lived episodes of myocardial ischaemia, a phenomenon termed 'ischaemic postconditioning' (IPost)[22,36,37]. The investigators found that applying three cycles of 30-s LAD occlusion and reflow within 1 min of myocardial reperfusion could reduce MI size by 44% in canine hearts[36]. In addition to its MI-limiting effects, IPost was found to confer a myriad of protective effects, including reduced levels of myocardial oedema, oxidative stress, and polymorphonuclear neutrophil accumulation, as well as preserved endothelial function. These findings were consistent with the reduced myocardial reperfusion injury seen in postconditioned hearts[36].

Interestingly, the concept of modifying reperfusion as a strategy to limit MI size had already been introduced in the 1980s as 'gentle'[38] or 'gradual'[39] reperfusion[40]. Furthermore, the term 'postconditioning' was coined several years earlier in 1996 by Na *et al.* to describe the phenomenon by which intermittent reperfusion — induced by ventricular premature beats — prevented reperfusion-induced ventricular fibrillation in ischaemic feline hearts.[41] Nevertheless, the concept of IPost has captured the

imagination and revitalized efforts to target myocardial reperfusion injury as a therapeutic strategy for reducing MI size. IPost has been shown to reduce MI size in rodents, rabbits, pigs, and other species, including humans, although the cardioprotective effect of IPost does not seem to be as robust as IPC[22, 23, 26, 42, 43].

[H2] Mechanisms of action

IPost seems to share some but not all of the signalling mechanisms recruited at the time of reperfusion by IPC(FIG. 2). Common signaling elements include activation of cell-surface receptors on the cardiomyocyte (such as adenosine, bradykinin, and opioids) and recruitment of prosurvival signalling pathways (such as RISK, SAFE, and cGMP), which mediate cardioprotection by preserving mitochondrial function (through reduced calcium overload, attenuated oxidative stress, and inhibited MPTP opening). Intermittent reperfusion induced by IPost has also been shown to delay restoration of intracellular pH, an effect that might contribute to the MPTP inhibition observed in postconditioned hearts[44,45]. Interestingly, the signalling pathways underlying IPost have been demonstrated to be species-specific, for example the RISK pathway mediates IPost in the hearts of rats, but not those of pigs[46]. The reasons for this difference are not clear. Notably, the importance of the RISK pathway for IPost has been demonstrated by using human atrial muscle harvested from patients undergoing CABG surgery[47].

[H2] IPost in STEMI

The ability to apply the therapeutic intervention at the onset of reperfusion in patients with STEMI has greatly facilitated the translation of IPost into the clinical setting. Only

2 years after the initial discovery of IPost, the first proof-of-concept clinical study was published[48]. In this study, IPost was performed after direct stenting within 1 min of reflow. Four episodes of 1 min inflation and 1 min deflation of an angioplasty balloon positioned upstream of the stent were performed. Investigators showed that this procedure reduced enzymatic MI size (assessed via total creatine kinase) by 36% and improved myocardial perfusion (assessed by myocardial blush grade) when compared to control[48]. In addition to providing the first evidence of successful translation of IPost into the clinical setting, findings from this study confirmed the existence of myocardial reperfusion injury in humans[49], because it clearly demonstrated that intervening at the onset of reperfusion reduces MI size.

Since publication of this first clinical study, investigators in a number of studies have used serum troponin release[50], myocardial single-photon emission computed tomography[51, 52], and cardiac MRI[53] to confirm the effects of IPost on limiting the size of an MI, and have demonstrated apparent long-term benefits on cardiac function[52]. However, other studies have failed to show a beneficial effect of IPost[54], and some researchers even report possible detrimental effects[55, 56] (TABLE 1). Although meta-analyses have confirmed the MI-limiting effects of IPost in patients with STEMI[57-59], the largest clinical study (which included 700 patients) showed no beneficial effect of IPost on ST-segment resolution, peak CK-MB levels, myocardial blush grade, or MACE at 30 days[60].

The reasons for the mixed results observed using IPost are not clear, but might be related to selection of patients and the IPost protocol itself (TABLE 1). In many of the studies showing positive results, patient selection criteria were meticulous (fully

occluded artery supplying a large area-at-risk in the absence of significant collaterals), although not all studies in which these selection criteria were applied showed positive effects. A study published in 2014 demonstrated that IPost was ineffective at reducing the size of MI in patients presenting with TIMI 2-3 flow, possibly because reperfusion had already spontaneously occurred. Patients most likely to benefit from IPost are those presenting with an occluded artery[61].

Another potential issue could be the presence of confounders, such as concomitant medications (morphine, nitrates, or $P2Y_{12}$ platelet inhibitors[62, 63, 64, 65]) and comorbidities (such as age, diabetes, hypertension, and hypercholesterolaemia), in patients with STEMI who received postconditioning. Interestingly, a retrospective analysis of 173 patients found that traditional cardiovascular risk factors in patients with STEMI — such as sex, diabetes, hypertension, dyslipidaemia, and obesity — did not affect cardioprotective efficacy of IPost[66]. However, these findings need to be confirmed in a larger, adequately powered, prospective study. In many of the studies showing positive effects, the IPost protocol was applied after direct stenting, upstream of the responsible lesion, thereby possibly avoiding coronary microembolization. By contrast, in many of the studies showing neutral or negative effects, the IPost protocol was performed after predilatation and at the site of the lesion. However, this alternative approach to the intervention was not used in all the IPost studies that showed neutral effects (TABLE 1). A further limitation to the interpretation of the findings regarding the use of IPost is that, given the nature of the protocol, it is not possible to blind the operator to the intervention.

Whether IPost can improve clinical outcomes is not known and is being investigated in the ongoing DANAMI-3 trial, the results of which will be available in early 2016[67]. Given the invasive nature of the IPost protocol and the mixed results of clinical studies so far, the translation of IPost for the benefit of patients with STEMI might prove to be difficult.

[H2] IPost in cardiac surgery

IPost has also been investigated in patients undergoing cardiac surgery, as a therapeutic strategy for protecting against perioperative myocardial injury caused by the acute global IRI that occurs when a patient is put onto and taken off cardio-pulmonary bypass. However, the protocol requires repeated cycles of clamping and unclamping the aorta. This procedure is performed three times for 30 s after a patient has been taken off bypass[68, 69]. Given the invasive nature of this IPost protocol and the potential risk of thromboembolic complications from manipulating an atherosclerotic aorta, the translation of IPost for adult patients undergoing cardiac surgery might not be possible. Notably, IPost could have greater therapeutic potential in children undergoing corrective cardiac surgery for congenital heart disease, where the risk of thromboembolism is substantially lower.

[H1] Pharmacological cardioprotection

The search for a pharmacological strategy to protect the heart against acute IRI preceded the discovery of IPC by many years. The elucidation of signalling pathways underlying ischaemic conditioning have resulted in advances in the understanding of

pathophysiological mechanisms of acute IRI and have identified a number of molecular targets amenable to pharmacological manipulation[56, 60].

The history of pharmacological cardioprotection has been disappointing. Anti-inflammatory agents, antioxidants, atorvastatin, calcium-channel blockers, erythropoietin, and magnesium have all been ineffective in reducing the size of MIs and improving clinical outcomes[70]. A number of pharmacological cardioprotective strategies, such as adenosine[71] and glucose–insulin–potassium therapy[72], showed mixed results, with cardioprotective efficacy depending on study design. More targeted pharmacological approaches have also failed to limit the size of MIs or improve clinical outcomes in clinical studies in which the cardioprotective effects of therapeutic hypothermia[73], agents targeting mitochondria (bendavia[74], cyclosporine A[1], TRO40303[75]), and modulation of nitric oxide signalling (using nitrite or inhaled nitric oxide)[76, 77] were investigated. Although cyclosporine A was shown to reduce the size of MIs in a proof-of-concept clinical study, it did not improve clinical outcomes in a subsequent large, multicentre clinical trial, the CIRCUS trial (reference 1), indicating the challenge of translating cardioprotection into clinical benefit. The reason for the failure of cyclosporine A to reduce MI size and improve clinical outcomes in patients with STEMI is not completely understood. Preclinical data are inconclusive, with some experimental studies failing to show a cardioprotective effect of cyclosporine A administered at reperfusion. Clinical data are limited, with only one study showing positive effects of cyclosporine A in patients with STEMI. Additional factors to consider are the use of the Ciclomulsion formulation of cyclosporine A, and the potential failure of cyclosporine A to reach its molecular target in time[11].

On a more optimistic note, a number of pharmacological strategies — such as the use of atrial natriuretic peptide, exenatide, or metoprolol — have been shown to reduce the size of an MI. Large, multicentre clinical studies are required to confirm these findings and to assess their effect on patient benefit (TABLE 2).

[H1] Remote ischaemic conditioning

A major disadvantage of IPC and IPost is that they both require an intervention to be applied directly to the heart, which is not always. Therefore, a strategy in which the cardioprotective stimulus is applied to an organ or tissue remote from the heart is a far more attractive clinical application.

In 1993, Przyklenk *et al.* made the crucial discovery that applying the IPC stimulus in one coronary vascular territory conferred tolerance to acute IRI in a different territory, suggesting that cardioprotection elicited by IPC could be transferred from one region of the heart to another[78]. This form of intramyocardial protection was later extended beyond the heart. Investigators reported that MI size could be reduced by inducing brief ischaemia and reperfusion to either the kidney[79] or small intestine[80] immediately before the sustained coronary artery occlusion. This phenomenon has been termed remote ischaemic conditioning (RIC)[81-85]. The concept of RIC has been further extended to encompass different organs and tissues, thereby providing a therapeutic strategy for inter-organ protection against the detrimental effects of acute IRI.

[H2] Mechanisms of action

The mechanism through which an episode of brief ischaemia and reperfusion in an organ or tissue located remotely from the heart exerts protection against a subsequently sustained insult of acute myocardial IRI is currently unclear. Experimental studies suggest that many of the underlying mechanistic pathways and signal transduction cascades activated within the protected organ might be similar to those recruited in the setting of IPC and IPost[81]. However, the mechanistic pathway that conveys the cardioprotective signal from the remote preconditioned organ or tissue to the heart remains uncertain.

Evidence indicates that a neurohumoral pathway is central to the protective effect underlying RIC. Reperfusion of the remote organ was found to be required for RIC cardioprotection, suggesting the 'washout' of a substance or humoral factor generated by the preconditioning ischaemia, which was then transported to the heart[79, 86]. In another study, blood harvested from a rabbit previously subjected to IPC of both the heart and kidney, reduced the size of MI when transfused into a IPC-naive rabbit[87], suggesting transfer of one or more humoral cardioprotective factors. Hexamethonium (a ganglion blocker)[86], resection of the neural innervation of the limb[88 89], genetic inhibition of preganglionic vagal neurons in the brainstem[90], and resection of the vagal nerve supply to the heart[91] have all been shown to abrogate the MI-limiting effects of limb RIC. These findings suggest that there is a requirement for an intact neural pathway to convey RIC cardioprotection, although the exact details of the neural pathway have not been completely elucidated. Stimulation of the neural pathway in the RIC-treated organ or tissue seems to be caused by local production of autacoids, such as adenosine[92, 93] and bradykinin[94]. Proteomic analysis of plasma harvested from RIC-treated animals has

not identified the cardioprotective factor, although evidence suggests that it is thermolabile, hydrophobic, and 3.0–8.5 kDa in size[95-99]. Other studies have provided experimental evidence implicating calcitonin-gene related peptide[100], stromal cell-derived factor 1[101], nitrite[102], and microRNA-144[103] as possible mediators of RIC cardioprotection, although conclusive evidence is lacking. A transferrable cardioprotective factor has been isolated from plasma harvested from animals and patients after a standard limb RIC protocol[93, 98, 104]. Generation of this factor has been shown to be dependent on an intact neural pathway to the RIC-treated limb[93]. Neural stimulation of the limb was achieved using direct nerve stimulation[105], electroacupuncture[106], topical capsaicin[105], or transcutaneous electrode stimulation[107] which generated a blood-borne transferrable cardioprotective factor and reduced size of MI in animal models. Finally, Jensen *et al.* confirmed the need for an intact neural pathway to the limb by showing that no blood-borne transferrable cardioprotective factor was produced when applying limb RIC to diabetic patients with a sensory neuropathy of the limb[108]. Further studies are required to tease out the exact interaction between the neuronal and humoral pathways underlying RIC, and to identify the blood-borne cardioprotective factor(s) which mediate RIC cardioprotection.

[H2] Clinical application

The discovery that cardioprotection could be elicited through applying the RIC stimulus to a limb[109, 110], by simply inflating and deflating a blood-pressure cuff placed on the upper arm or thigh[111], has facilitated the translation of RIC into the clinical setting. The limb RIC stimulus itself has not been fully characterized. Experimental animal models

most often use 5–15 min; however, the most effective limb RIC stimulus for experimental or clinical settings remains unclear. The ability to deliver the stimulus noninvasively to the limb has allowed RIC to be delivered at different time-points with respect to the ischaemia–reperfusion insult. RIC can be delivered 24 h (delayed remote ischaemic preconditioning or RIPC), or immediately before the index ischaemia (RIPC), after the onset of index ischaemia, but before reperfusion (remote ischaemic perconditioning or RIPerC)[112], at the onset of reperfusion (remote ischaemic postconditioning or RIPost)[113, 114], or even 15–30 min into reperfusion (delayed RIPost)[115]. This flexibility in timing of the RIC stimulus has enabled its application in a wide variety of clinical settings of acute IRI (FIG. 1).

[H3] RIC in cardiac surgery

In 2000, Guanydin *et al.* published the first study of the effect of limb RIC in patients undergoing cardiac surgery, although in this small study of eight patients, perioperative myocardial injury (PMI) was not assessed[116]. In 2006, Cheung *et al.* published the first study to demonstrate a cardioprotective effect of limb RIC[117]. The study involved children undergoing cardiac surgery for congenital heart disease. Four 5-min cycles of lower limb ischaemia and reperfusion, induced by inflating and deflating a blood-pressure cuff placed on the thigh, reduced PMI (as indicated by serum troponin I level) and requirement for inotropes, and decreased airway pressure. Similar beneficial effects were reported in adults undergoing CABG surgery, with a 43% reduction in PMI (assessed via the 72 h area under the curve for troponin T level)[118]. However, although a number of studies have confirmed the beneficial effects of RIC in patients undergoing

cardiac surgery in terms of attenuating PMI, a substantial number of studies have provided neutral findings (TABLE 3). Meta-analyses seemed to confirm the cardioprotective effect of limb RIC in terms of reducing PMI[119-122], whereas several large, prospective, multicentre, randomized clinical trials (adequately powered to detect major adverse cardiovascular events) published in the past 2 years showed that limb RIC has no beneficial effects on major clinical outcomes in patients undergoing cardiac surgery[123-126] (TABLE 3).

The reasons why limb RIC is not beneficial in patients undergoing cardiac surgery are multiple and complex (TABLE 3). Experimental data have established that RIC is most effective at protecting the heart against acute IRI. Therefore, cardiac surgery might not be the optimal setting for investigating cardioprotective therapies (because the causes of PMI are multiple). Furthermore, given that myocardial protection strategies are already being used during surgery, the magnitude of PMI is relatively small (when compared to STEMI), making it difficult to demonstrate an additional cardioprotective effect. Furthermore, the optimal surgical setting for testing RIC is not known. Whether studies should have been restricted to CABG surgery alone (where acute IRI possibly has the major role in PMI) — and valve or aortic surgery (where the causes of PMI also include direct injury to the myocardium) should have been excluded — is not clear.

The most effective RIC protocol is yet to be defined. The protocol most often used in studies (four 5-min cycles of limb ischaemia–reperfusion) has been poorly characterized in both animal and clinical studies. Furthermore, whether RIC is more effective if delivered before or after surgical incision is not clear. The blinding of the

investigators to the RIC protocol might have been suboptimal in many of the clinical studies reporting positive results[127]. Most studies achieved only incomplete blinding using a deflated cuff, instead of full blinding using a 'dummy arm'[128].

Comorbidities in patients undergoing cardiac surgery (such as age, diabetes, obesity, hypertension, hypercholesterolaemia) have been shown in animal studies to affect endogenous cardioprotective strategies such as IPC and IPost; their effect on RIC, however, has not been well defined[63]. Another consideration is that patients undergoing cardiac surgery receive a variety of drugs that can potentially affect the cardioprotective efficacy of RIC. These drugs include anaesthetics (volatile anaesthetic agents [isoflurane, sevoflurane] and propofol), analgesics (morphine), and others such as nitrates. Data from some studies suggest that RIC might be ineffective in the presence of isoflurane[129] or propofol[130]; however, no clear association exists between the use of isoflurane or propofol and study outcome. The majority of smaller studies documenting a cardioprotective effect showed that RIC reduces the extent of PMI defined as reduction in either peak or area-under-the-curve levels of serum cardiac enzymes. However, the magnitude of this cardioprotective effect was smaller in larger studies[131, 132] and absent in the multicentre clinical outcomes trials[124-126]. Few studies have shown a significant reduction in the incidence of CABG-related MI, as defined in recent clinical guidelines (termed Type 5 MI)[133], an end point that is a critical determinant of clinical outcomes after surgery[6].

A single reason for RIC not being of benefit in the setting of cardiac surgery might not become apparent. All the above factors probably contributed, highlighting the

difficulties of translating a promising cardioprotective therapy into the clinical setting for patient benefit.

[H3] RIC in planned PCI

RIC has been investigated as a cardioprotective strategy in patients undergoing planned PCI. PMI occurs in ~30% of stable patients undergoing planned PCI and in up to 80% of unstable patients undergoing urgent PCI. PMI can be quantified by measuring the release of serum cardiac enzymes during PCI[134]. However, the aetiology of PCI-related myocardial injury is not due to acute IRI *per se*, but is mainly caused by acute ischaemic injury (arising from distal branch occlusions, and coronary embolization). Such complications can occur particularly after multivessel and complex PCI[134]. The first investigation of limb RIC in this clinical setting was published by Iliodromitis *et al.* in 2006; they showed in a study including 41 patients that RIC using bilateral upper arm cuff inflations and deflations exacerbated myocardial injury[135]. In a subsequent study published in 2010, which was larger in size, Hoole *et al.* found that this intervention reduced the magnitude of PCI-related myocardial injury[136]. After these early studies, a number of confirmatory studies have been published, although other studies have provided neutral results (TABLE 4).

The reasons for these discrepancies are not clear, but several factors possibly contribute. RIC has been shown to protect mainly against acute IRI, which is not a major component of PCI-related myocardial injury. Moreover, compared with stable patients, unstable patients might not benefit from RIC, because they could have been preconditioned by anginal chest pain. Comorbidities and concomitant medication might

also affect RIC cardioprotection. Finally, whether simple versus multivessel or complex PCI is more amenable to RIC is not clear. The presence of predilatation or postdilatation during PCI may have attenuated any cardioprotetctive effect elicited by RIC.

Meta-analyses have indicated that limb RIC reduces the magnitude of PCI-related myocardial injury and decreases the incidence of PCI-related MI in stable patients undergoing PCI[119, 137]. Further multicentre studies are required to confirm these findings and determine whether this therapeutic approach can actually reduce major adverse cardiac events in patients undergoing planned PCI.

Compared with CABG surgery and PCI, the clinical setting of STEMI provides the 'purest' example of acute myocardial IRI and best reflects the pre-clinical animal models of acute myocardial IRI. Therefore, the potentially cardioprotective therapy of RIC may be best suited to patients with STEMI undergoing PPCI, especially as it can be applied in this setting to target myocardial reperfusion injury specifically. Several proof-of-concept studies have reported cardioprotective effects of limb RIC in patients with STEMI treated with PPCI (TABLE 5). RIC seemed to be effective when given in the ambulance by paramedics[138], on arrival at the hospital before PPCI[139, 140], and even at the onset of reperfusion at the time of PPCI[141]. Whether limb RIC can improve clinical outcomes in patients undergoing PPCI is currently being investigated in the CONDI2/ERIC-PPCI trial (NCT01857414). The primary outcomes of this study is to determine whether RIC can reduce rates of cardiac death and hospitalization for heart failure at 12 months.

A more effective approach for targeting myocardial reperfusion injury might be to combine therapeutic interventions. This strategy has been shown to have an additive

effect on reduction in size of MI in experimental studies[142]. Initial clinical studies have found that this approach might have potential as a therapy. The combination of RIC with IPost has been shown to be more effective than IPost alone[143] (TABLES 1 and 5).

[H3] Other clinical settings

The heart is subjected to acute global IRI in cardiac transplantation and in cardiac arrest providing further opportunities to investigate the cardioprotective effect of limb RIC. This approach has been shown to be promising in experimental studies[144]. In particular, because there is a risk of multiorgan dysfunction arising from acute IRI in these patients, limb RIC might have the additional benefit of protecting noncardiac organs and tissues.

To date, RIC has been investigated as a one-off application; however, cumulative benefits might be accrued with repeated RIC stimuli. One experimental study demonstrated that repeating limb RIC daily for 28 days prevented adverse LV remodelling after an MI in rat hearts[145]. Whether repeated episodes of limb RIC, applied as a daily therapy, are beneficial in the clinical setting is not known. Interestingly, given that exercise has also been reported to induce cardioprotection, a parallel might exist between exercise and daily RIC as a cardioprotective strategy[146]. Two clinical studies are currently underway to investigate the effect of daily RIC for 4 weeks on LV remodelling after an MI: the DREAM (NCT01664611) CRIC-RCT (NCT01817114) trials. The CONDI-HF study (NCT02248441) is ongoing to assess the effect of daily RIC on LV ejection fraction in patients with chronic heart failure.

[H1] Challenges facing clinical research

Although the concept of IPC was first described in 1986, the therapeutic potential of ischaemic conditioning has been realized in only the past 5–10 years, and whether the intervention can improve clinical outcomes still remains to be determined. A vast number of novel cardioprotective therapies with efficacy proven in experimental animal studies have failed to improve clinical outcomes in patients. The reasons for the failure to translate the cardioprotective effects of ischaemic conditioning strategies from the bench to bedside have been extensively discussed in the literature[147-151]. Briefly, this failure can be attributed to several factors. The available animal models of acute IRI are inadequate in representing the wide spectrum of comorbidities and coexisting conditions of patients with IHD (such as advanced age, diabetes, hypertension, hyperlipidaemia, other medical therapy, and pre-existing coronary artery disease)[63]. Moreover, some clinical studies were poorly designed, and investigators failed to take into account the results from experimental studies[70, 152]. Many novel cardioprotective therapies have been investigated in the clinical setting without thorough testing in preclinical animal models. Therefore, after two National Heart, Lung, and Blood Institute workshops to discuss this issue, the Consortium for preclinicAl assESsment of cARdioprotective therapies (CAESAR) was formed. The objective of this consortium was to enable testing of novel cardioprotective therapies using small-animal and large-animal models of MI within a network of centres, an approach similar to a multicentre, randomized, controlled clinical trial[153, 154]. In addition, guidelines for the future design of both basic science and clinical studies for the assessment of novel cardioprotective therapies have been proposed[70, 151, 152].

The major challenge facing clinical cardioprotection research is that clinical outcomes of patients with STEMI after PPCI continue to improve, making it increasingly difficult to demonstrate a reduction in size of MI and improvement in clinical outcomes with a novel cardioprotective therapy. However, although mortality after STEMI is declining, the number of patients surviving a STEMI and subsequently developing heart failure is increasing. Therefore, novel therapeutic strategies that can prevent myocardial reperfusion injury and reduce the size of MI, to preserve LV systolic function and prevent onset of heart failure, are still needed. A similar challenge faces clinical research on cardioprotection in patients undergoing CABG surgery. Improvements in surgical techniques and advances in myocardial protection have reduced the extent of PMI together with patient mortality. Currently, the death rate at 1 year after isolated CABG surgery is as low as 1–2%. However, with an ageing population and increasing prevalence of comorbidities, such as diabetes, obesity, and hypertension, novel cardioprotective therapies for high-risk patients will be required. Therefore, patients at high-risk of operative complications should perhaps be the focus of future clinical cardioprotection studies.

[H1] Conclusions

Ischaemic conditioning offers a powerful endogenous cardioprotective strategy for reducing size of MI in patients with STEMI undergoing reperfusion, and attenuating perioperative and periprocedural myocardial injury in patients undergoing CABG surgery or PCI, respectively. The different forms of ischaemic conditioning enable its application in a number of clinical settings (FIG. 1). In particular, the simplicity and

noninvasive nature, as well as the flexibility of the timing of the RIC stimulus, make it feasible to apply in many clinical scenarios involving acute IRI. Clearly, ischaemic conditioning is highly cardioprotective, as shown by the wealth of preclinical data from animal experiments. However, most importantly, clinical studies have produced mixed results. The most promising data exist for limb RIC in patients with STEMI Results from clinical studies of pharmacological cardioprotection strategies have been generally disappointing to date. Promising agents include exenatide and metoprolol, although large, multicentre studies are required to confirm their cardioprotective potential and to determine whether they can improve clinical outcomes. Although clinical cardioprotection research has been challenging, novel therapies are still needed because of the increasing prevalence of heart failure in patients with IHD. Further work is required to optimize the design of our experimental animal and clinical studies, and improve how we select which novel cardioprotective therapy to test in a clinical setting. Such advances could facilitate the discovery of new effective therapies for reducing MI size and improving clinical outcomes in patients with IHD.

1. Cung,T.T. *et al.* Cyclosporine before PCI in Patients with Acute Myocardial Infarction. *N. Engl. J. Med.* **373**, 1021-1031 (2015).
2. Venugopal,V., Ludman,A., Yellon,D.M., & Hausenloy,D.J. 'Conditioning' the heart during surgery. *Eur. J. Cardiothorac. Surg.* **35**, 977-987 (2009).
3. Hausenloy,D.J., Boston-Griffiths,E., & Yellon,D.M. Cardioprotection during cardiac surgery. *Cardiovasc. Res.* **94**, 253-265 (2012).
4. Kathiresan,S. *et al.* Cardiac troponin T elevation after coronary artery bypass grafting is associated with increased one-year mortality. *Am. J. Cardiol.* **94**, 879-881 (2004).
5. Croal,B.L. *et al.* Relationship between postoperative cardiac troponin I levels and outcome of cardiac surgery. *Circulation* **114**, 1468-1475 (2006).
6. Wang,T.K. *et al.* Diagnosis of MI after CABG with high-sensitivity troponin T and new ECG or echocardiogram changes: relationship with mortality and

validation of the universal definition of MI. *Eur. Heart J. Acute. Cardiovasc. Care* **2**, 323-333 (2013).
7. Braunwald,E. & Kloner,R.A. Myocardial reperfusion: a double-edged sword? *J. Clin. Invest* **76**, 1713-1719 (1985).
8. Piper,H.M., Garcia-Dorado,D., & Ovize,M. A fresh look at reperfusion injury. *Cardiovasc. Res.* **38**, 291-300 (1998).
9. Yellon,D.M. & Hausenloy,D.J. Myocardial reperfusion injury. *N. Engl. J Med.* **357**, 1121-1135 (2007).
10. Hausenloy,D.J. & Yellon,D.M. Time to take myocardial reperfusion injury seriously. *N. Engl. J Med.* **359**, 518-520 (2008).
11. Hausenloy,D.J. & Yellon,D.M. Targeting Myocardial Reperfusion Injury--The Search Continues. *N. Engl. J. Med.* **373**, 1073-1075 (2015).
12. Jennings,R.B., SOMMERS,H.M., SMYTH,G.A., FLACK,H.A., & LINN,H. Myocardial necrosis induced by temporary occlusion of a coronary artery in the dog. *Arch. Pathol.* **70**, 68-78 (1960).
13. Krug,A., Du Mesnil,d.R., & Korb,G. Blood supply of the myocardium after temporary coronary occlusion. *Circ. Res.* **19**, 57-62 (1966).
14. Kloner,R.A., Ganote,C.E., & Jennings,R.B. The "no-reflow" phenomenon after temporary coronary occlusion in the dog. *J Clin. Invest* **54**, 1496-1508 (1974).
15. Galiuto,L. & Crea,F. No-reflow: a heterogeneous clinical phenomenon with multiple therapeutic strategies. *Curr. Pharm. Des* **12**, 3807-3815 (2006).
16. White,S.K., Hausenloy,D.J., & Moon,J.C. Imaging the myocardial microcirculation post-myocardial infarction. *Curr. Heart Fail. Rep.* **9**, 282-292 (2012).
17. Bogaert,J., Kalantzi,M., Rademakers,F.E., Dymarkowski,S., & Janssens,S. Determinants and impact of microvascular obstruction in successfully reperfused ST-segment elevation myocardial infarction. Assessment by magnetic resonance imaging. *Eur. Radiol.* **17**, 2572-2580 (2007).
18. Ong,S.B., Samangouei,P., Kalkhoran,S.B., & Hausenloy,D.J. The mitochondrial permeability transition pore and its role in myocardial ischemia reperfusion injury. *J. Mol. Cell Cardiol.* **78C**, 23-34 (2015).
19. Murry,C.E., Jennings,R.B., & Reimer,K.A. Preconditioning with ischemia: a delay of lethal cell injury in ischemic myocardium. *Circulation* **74**, 1124-1136 (1986).
20. Reimer,K.A., Murry,C.E., Yamasawa,I., Hill,M.L., & Jennings,R.B. Four brief periods of myocardial ischemia cause no cumulative ATP loss or necrosis. *Am. J. Physiol* **251**, H1306-H1315 (1986).
21. Yellon,D.M. & Downey,J.M. Preconditioning the myocardium: from cellular physiology to clinical cardiology. *Physiol Rev.* **83**, 1113-1151 (2003).
22. Hausenloy,D.J. Cardioprotection techniques: preconditioning, postconditioning and remote con-ditioning (basic science). *Curr. Pharm. Des* **19**, 4544-4563 (2013).
23. Bulluck,H. & Hausenloy,D.J. Ischaemic conditioning: are we there yet? *Heart* **101**, 1067-1077 (2015).

24. Kuzuya,T. *et al.* Delayed effects of sublethal ischemia on the acquisition of tolerance to ischemia. *Circ. Res.* **72**, 1293-1299 (1993).
25. Marber,M.S., Latchman,D.S., Walker,J.M., & Yellon,D.M. Cardiac stress protein elevation 24 hours after brief ischemia or heat stress is associated with resistance to myocardial infarction. *Circulation* **88**, 1264-1272 (1993).
26. Heusch,G. Molecular basis of cardioprotection: signal transduction in ischemic pre-, post-, and remote conditioning. *Circ. Res.* **116**, 674-699 (2015).
27. Hausenloy,D.J. & Yellon,D.M. The second window of preconditioning (SWOP) where are we now? *Cardiovasc. Drugs Ther.* **24**, 235-254 (2010).
28. Hausenloy,D.J. & Yellon,D.M. Reperfusion injury salvage kinase signalling: taking a RISK for cardioprotection. *Heart Fail. Rev.* **12**, 217-234 (2007).
29. Hausenloy,D.J., Lecour,S., & Yellon,D.M. Reperfusion injury salvage kinase and survivor activating factor enhancement prosurvival signaling pathways in ischemic postconditioning: two sides of the same coin. *Antioxid. Redox. Signal.* **14**, 893-907 (2011).
30. Ong,S.B., Dongworth,R.K., Cabrera-Fuentes,H.A., & Hausenloy,D.J. Role of the MPTP in conditioning the heart - translatability and mechanism. *Br. J. Pharmacol.* **172**, 2074-2084 (2015).
31. Varga,Z.V. *et al.* Functional Genomics of Cardioprotection by Ischemic Conditioning and the Influence of Comorbid Conditions: Implications in Target Identification. *Curr. Drug Targets.* **16**, 904-911 (2015).
32. Williams,R.P., Manou-Stathopoulou,V., Redwood,S.R., & Marber,M.S. 'Warm-up Angina': harnessing the benefits of exercise and myocardial ischaemia. *Heart* **100**, 106-114 (2014).
33. Eitel,I. & Thiele,H. Cardioprotection by pre-infarct angina: training the heart to enhance myocardial salvage. *Eur. Heart J. Cardiovasc. Imaging* **14**, 1115-1116 (2013).
34. Yellon,D.M., Alkhulaifi,A.M., & Pugsley,W.B. Preconditioning the human myocardium. *Lancet* **342**, 276-277 (1993).
35. Walsh,S.R. *et al.* Ischaemic preconditioning during cardiac surgery: systematic review and meta-analysis of perioperative outcomes in randomised clinical trials. *Eur. J. Cardiothorac. Surg.* **34**, 985-994 (2008).
36. Zhao,Z.Q. *et al.* Inhibition of myocardial injury by ischemic postconditioning during reperfusion: comparison with ischemic preconditioning. *Am. J. Physiol Heart Circ. Physiol* **285**, H579-H588 (2003).
37. Vinten-Johansen,J. & Shi,W. The science and clinical translation of remote postconditioning. *J. Cardiovasc. Med. (Hagerstown.)* **14**, 206-213 (2013).
38. Okamoto,F., Allen,B.S., Buckberg,G.D., Bugyi,H., & Leaf,J. Reperfusion conditions: importance of ensuring gentle versus sudden reperfusion during relief of coronary occlusion. *J. Thorac. Cardiovasc Surg.* **92**, 613-620 (1986).
39. Sato,H., Jordan,J.E., Zhao,Z.Q., Sarvotham,S.S., & Vinten-Johansen,J. Gradual reperfusion reduces infarct size and endothelial injury but augments neutrophil accumulation. *Ann. Thorac. Surg.* **64**, 1099-1107 (1997).

40. Heusch,G. Postconditioning: old wine in a new bottle? *J Am. Coll. Cardiol.* **44**, 1111-1112 (2004).
41. Na,H.S. *et al.* Ventricular premature beat-driven intermittent restoration of coronary blood flow reduces the incidence of reperfusion-induced ventricular fibrillation in a cat model of regional ischemia. *Am. Heart J* **132**, 78-83 (1996).
42. Tsang,A., Hausenloy,D.J., & Yellon,D.M. Myocardial postconditioning: reperfusion injury revisited. *Am J Physiol Heart Circ. Physiol* **289**, H2-H7 (2005).
43. Shi,W. & Vinten-Johansen,J. Endogenous cardioprotection by ischaemic postconditioning and remote conditioning. *Cardiovasc. Res.* **94**, 206-216 (2012).
44. Fujita,M. *et al.* Prolonged transient acidosis during early reperfusion contributes to the cardioprotective effects of postconditioning. *Am J Physiol Heart Circ. Physiol* **292**, H2004-H2008 (2007).
45. Cohen,M.V., Yang,X.M., & Downey,J.M. The pH hypothesis of postconditioning: staccato reperfusion reintroduces oxygen and perpetuates myocardial acidosis. *Circulation* **115**, 1895-1903 (2007).
46. Skyschally,A. *et al.* Across-Species Transfer of Protection by Remote Ischemic Preconditioning With Species-Specific Myocardial Signal Transduction by Reperfusion Injury Salvage Kinase and Survival Activating Factor Enhancement Pathways. *Circ. Res.* **117**, 279-288 (2015).
47. Sivaraman,V. *et al.* Postconditioning protects human atrial muscle through the activation of the RISK pathway. *Basic Res. Cardiol* **102**, 453-459 (2007).
48. Staat,P. *et al.* Postconditioning the human heart. *Circulation* **112**, 2143-2148 (2005).
49. Yellon,D.M. & Opie,L.H. Postconditioning for protection of the infarcting heart. *Lancet* **367**, 456-458 (2006).
50. Ma,X., Zhang,X., Li,C., & Luo,M. Effect of Postconditioning on Coronary Blood Flow Velocity and Endothelial Function and LV Recovery After Myocardial Infarction. *J. Interv. Cardiol.* **19**, 367-375 (2006).
51. Yang,X.C. *et al.* Reduction in myocardial infarct size by postconditioning in patients after percutaneous coronary intervention. *J Invasive. Cardiol* **19**, 424-430 (2007).
52. Thibault,H. *et al.* Long-term benefit of postconditioning. *Circulation* **117**, 1037-1044 (2008).
53. Lonborg,J. *et al.* Cardioprotective effects of ischemic postconditioning in patients treated with primary percutaneous coronary intervention, evaluated by magnetic resonance. *Circ. Cardiovasc. Interv.* **3**, 34-41 (2010).
54. Sorensson,P. *et al.* Effect of postconditioning on infarct size in patients with ST elevation myocardial infarction. *Heart* **96**, 1710-1715 (2010).
55. Freixa,X. *et al.* Ischaemic postconditioning revisited: lack of effects on infarct size following primary percutaneous coronary intervention. *Eur Heart J* **33**, 103-112 (2012).
56. Tarantini,G. *et al.* Postconditioning during coronary angioplasty in acute myocardial infarction: the POST-AMI trial. *Int. J. Cardiol.* **162**, 33-38 (2012).

57. Favaretto,E. *et al.* Meta-Analysis of Randomized Trials of Postconditioning in ST-Elevation Myocardial Infarction. *Am. J. Cardiol.* **114**, 946-952 (2014).
58. Khalili,H. *et al.* Surrogate and clinical outcomes following ischemic postconditioning during primary percutaneous coronary intervention of ST--segment elevation myocardial infarction: a meta-analysis of 15 randomized trials. *Catheter. Cardiovasc. Interv.* **84**, 978-986 (2014).
59. Touboul,C. *et al.* Ischaemic postconditioning reduces infarct size: systematic review and meta-analysis of randomized controlled trials. *Arch. Cardiovasc. Dis.* **108**, 39-49 (2015).
60. Hahn,J.Y. *et al.* Ischemic postconditioning during primary percutaneous coronary intervention: the effects of postconditioning on myocardial reperfusion in patients with ST-segment elevation myocardial infarction (POST) randomized trial. *Circulation* **128**, 1889-1896 (2013).
61. Roubille,F. *et al.* No post-conditioning in the human heart with thrombolysis in myocardial infarction flow 2-3 on admission. *Eur. Heart J.* **35**, 1675-1682 (2014).
62. Ferdinandy,P., Szilvassy,Z., & Baxter,G.F. Adaptation to myocardial stress in disease states: is preconditioning a healthy heart phenomenon? *Trends Pharmacol. Sci.* **19**, 223-229 (1998).
63. Ferdinandy,P., Hausenloy,D.J., Heusch,G., Baxter,G.F., & Schulz,R. Interaction of Risk Factors, Comorbidities, and Comedications with Ischemia/Reperfusion Injury and Cardioprotection by Preconditioning, Postconditioning, and Remote Conditioning. *Pharmacol. Rev.* **66**, 1142-1174 (2014).
64. Roubille,F. *et al.* Cardioprotection by clopidogrel in acute ST-elevated myocardial infarction patients: a retrospective analysis. *Basic Res. Cardiol.* **107**, 275 (2012).
65. Yang,X.M. *et al.* Platelet P2Y(1)(2) blockers confer direct postconditioning-like protection in reperfused rabbit hearts. *J. Cardiovasc. Pharmacol. Ther.* **18**, 251-262 (2013).
66. Pichot,S. *et al.* Influence of cardiovascular risk factors on infarct size and interaction with mechanical ischaemic postconditioning in ST-elevation myocardial infarction. *Open. Heart* **2**, e000175 (2015).
67. Hofsten,D.E. *et al.* The Third DANish Study of Optimal Acute Treatment of Patients with ST-segment Elevation Myocardial Infarction: Ischemic postconditioning or deferred stent implantation versus conventional primary angioplasty and complete revascularization versus treatment of culprit lesion only: Rationale and design of the DANAMI 3 trial program. *Am. Heart J.* **169**, 613-621 (2015).
68. Luo,W., Li,B., Lin,G., & Huang,R. Postconditioning in cardiac surgery for tetralogy of Fallot. *J Thorac. Cardiovasc Surg.* **133**, 1373-1374 (2007).
69. Luo,W., Li,B., Chen,R., Huang,R., & Lin,G. Effect of ischemic postconditioning in adult valve replacement. *Eur. J Cardiothorac. Surg.* **33**, 203-208 (2008).

70. Hausenloy,D.J. *et al.* Translating cardioprotection for patient benefit: position paper from the Working Group of Cellular Biology of the Heart of the European Society of Cardiology. *Cardiovasc. Res.* **98**, 7-27 (2013).
71. Kloner,R.A. *et al.* Impact of time to therapy and reperfusion modality on the efficacy of adenosine in acute myocardial infarction: the AMISTAD-2 trial. *Eur. Heart J* **27**, 2400-2405 (2006).
72. Mehta,S.R. *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* **293**, 437-446 (2005).
73. Erlinge,D. *et al.* Therapeutic hypothermia for the treatment of acute myocardial infarction-combined analysis of the RAPID MI-ICE and the CHILL-MI trials. *Ther. Hypothermia. Temp. Manag.* **5**, 77-84 (2015).
74. Chakrabarti,A.K. *et al.* Rationale and design of the EMBRACE STEMI study: a phase 2a, randomized, double-blind, placebo-controlled trial to evaluate the safety, tolerability and efficacy of intravenous Bendavia on reperfusion injury in patients treated with standard therapy including primary percutaneous coronary intervention and stenting for ST-segment elevation myocardial infarction. *Am. Heart J.* **165**, 509-514 (2013).
75. Atar,D. *et al.* Effect of intravenous TRO40303 as an adjunct to primary percutaneous coronary intervention for acute ST-elevation myocardial infarction: MITOCARE study results. *Eur. Heart J.* **36**, 112-119 (2015).
76. Siddiqi,N. *et al.* Intravenous sodium nitrite in acute ST-elevation myocardial infarction: a randomized controlled trial (NIAMI). *Eur. Heart J.* **35**, 1255-1262 (2014).
77. Jones,D.A. *et al.* Randomized phase 2 trial of intracoronary nitrite during acute myocardial infarction. *Circ. Res.* **116**, 437-447 (2015).
78. Przyklenk,K., Bauer,B., Ovize,M., Kloner,R.A., & Whittaker,P. Regional ischemic 'preconditioning' protects remote virgin myocardium from subsequent sustained coronary occlusion. *Circulation* **87**, 893-899 (1993).
79. McClanahan,T., Nao,B., Wolke,L., Martin BJ, & Mezt TE. Brief renal occlusion and reperfusion reduces myocardial infarct size in rabbits. FASEB J 7, A18. 1993.
80. Gho,B.C., Schoemaker,R.G., van den Doel,M.A., Duncker,D.J., & Verdouw,P.D. Myocardial protection by brief ischemia in noncardiac tissue. *Circulation* **94**, 2193-2200 (1996).
81. Hausenloy,D.J. & Yellon,D.M. Remote ischaemic preconditioning: underlying mechanisms and clinical application. *Cardiovasc Res.* **79**, 377-386 (2008).
82. Candilio,L., Hausenloy,D.J., & Yellon,D.M. Remote ischemic conditioning: a clinical trial's update. *J. Cardiovasc. Pharmacol. Ther.* **16**, 304-312 (2011).
83. Pickard,J.M. *et al.* Remote ischemic conditioning: from experimental observation to clinical application: report from the 8th Biennial Hatter Cardiovascular Institute Workshop. *Basic Res. Cardiol.* **110**, 453 (2015).
84. Sivaraman,V., Pickard,J.M., & Hausenloy,D.J. Remote ischaemic conditioning: cardiac protection from afar. *Anaesthesia* **70**, 732-748 (2015).

85. Heusch,G., Botker,H.E., Przyklenk,K., Redington,A., & Yellon,D. Remote Ischemic Conditioning. *J. Am. Coll. Cardiol.* **65**, 177-195 (2015).
86. Gho,B.C., Schoemaker,R.G., van den Doel,M.A., Duncker,D.J., & Verdouw,P.D. Myocardial protection by brief ischemia in noncardiac tissue. *Circulation* **94**, 2193-2200 (1996).
87. Dickson,E.W. *et al.* Ischemic preconditioning may be transferable via whole blood transfusion: preliminary evidence. *J Thromb. Thrombolysis.* **8**, 123-129 (1999).
88. Ding,Y.F., Zhang,M.M., & He,R.R. Role of renal nerve in cardioprotection provided by renal ischemic preconditioning in anesthetized rabbits. *Sheng Li Xue. Bao.* **53**, 7-12 (2001).
89. Lim,S.Y., Yellon,D.M., & Hausenloy,D.J. The neural and humoral pathways in remote limb ischemic preconditioning. *Basic Res. Cardiol* **105**, 651-655 (2010).
90. Mastitskaya,S. *et al.* Cardioprotection evoked by remote ischaemic preconditioning is critically dependent on the activity of vagal pre-ganglionic neurones. *Cardiovasc. Res.* **95**, 487-494 (2012).
91. Donato,M. *et al.* Role of the parasympathetic nervous system in cardioprotection by remote hindlimb ischaemic preconditioning. *Exp. Physiol* **98**, 425-434 (2013).
92. Liem,D.A., Verdouw,P.D., Ploeg,H., Kazim,S., & Duncker,D.J. Sites of action of adenosine in interorgan preconditioning of the heart. *Am J Physiol Heart Circ. Physiol* **283**, H29-H37 (2002).
93. Steensrud,T. *et al.* Pretreatment with the nitric oxide donor SNAP or nerve transection blocks humoral preconditioning by remote limb ischemia or intra-arterial adenosine. *Am. J. Physiol Heart Circ. Physiol* **299**, H1598-H1603 (2010).
94. Schoemaker,R.G. & van Heijningen,C.L. Bradykinin mediates cardiac preconditioning at a distance. *Am. J. Physiol Heart Circ. Physiol* **278**, H1571-H1576 (2000).
95. Dickson,E.W. *et al.* Naloxone blocks transferred preconditioning in isolated rabbit hearts. *J Mol. Cell Cardiol* **33**, 1751-1756 (2001).
96. Lang,S.C. *et al.* Myocardial preconditioning and remote renal preconditioning--identifying a protective factor using proteomic methods? *Basic Res. Cardiol* **101**, 149-158 (2006).
97. Serejo,F.C., Rodrigues,L.F., Jr., Silva Tavares,K.C., de Carvalho,A.C., & Nascimento,J.H. Cardioprotective properties of humoral factors released from rat hearts subject to ischemic preconditioning. *J Cardiovasc Pharmacol.* **49**, 214-220 (2007).
98. Shimizu,M. *et al.* Transient limb ischaemia remotely preconditions through a humoral mechanism acting directly on the myocardium: evidence suggesting cross-species protection. *Clin. Sci. (Lond)* **117**, 191-200 (2009).
99. Breivik,L., Helgeland,E., Aarnes,E.K., Mrdalj,J., & Jonassen,A.K. Remote postconditioning by humoral factors in effluent from ischemic preconditioned

rat hearts is mediated via PI3K/Akt-dependent cell-survival signaling at reperfusion. *Basic Res. Cardiol* **106**, 135-145 (2011).
100. Wolfrum,S. *et al.* Calcitonin gene related peptide mediates cardioprotection by remote preconditioning. *Regul. Pept.* **127**, 217-224 (2005).
101. Davidson,S.M. *et al.* Remote ischaemic preconditioning involves signalling through the SDF-1alpha/CXCR4 signalling axis. *Basic Res. Cardiol.* **108**, 377 (2013).
102. Rassaf,T. *et al.* Circulating nitrite contributes to cardioprotection by remote ischemic preconditioning. *Circ. Res.* **114**, 1601-1610 (2014).
103. Li,J. *et al.* MicroRNA-144 is a circulating effector of remote ischemic preconditioning. *Basic Res. Cardiol.* **109**, 423 (2014).
104. Wang,L. *et al.* Remote ischemic preconditioning elaborates a transferable blood-borne effector that protects mitochondrial structure and function and preserves myocardial performance after neonatal cardioplegic arrest. *J Thorac. Cardiovasc. Surg.* **136**, 335-342 (2008).
105. Redington,K.L. *et al.* Remote cardioprotection by direct peripheral nerve stimulation and topical capsaicin is mediated by circulating humoral factors. *Basic Res. Cardiol* **107**, 1-10 (2012).
106. Redington,K.L. *et al.* Electroacupuncture reduces myocardial infarct size and improves post-ischemic recovery by invoking release of humoral, dialyzable, cardioprotective factors. *J. Physiol Sci.* **63**, 219-223 (2013).
107. Merlocco,A.C. *et al.* Transcutaneous electrical nerve stimulation as a novel method of remote preconditioning: in vitro validation in an animal model and first human observations. *Basic Res. Cardiol.* **109**, 406 (2014).
108. Jensen,R.V., Stottrup,N.B., Kristiansen,S.B., & Botker,H.E. Release of a humoral circulating cardioprotective factor by remote ischemic preconditioning is dependent on preserved neural pathways in diabetic patients. *Basic Res. Cardiol.* **107**, 285 (2012).
109. Birnbaum,Y., Hale,S.L., & Kloner,R.A. Ischemic preconditioning at a distance: reduction of myocardial infarct size by partial reduction of blood supply combined with rapid stimulation of the gastrocnemius muscle in the rabbit. *Circulation* **96**, 1641-1646 (1997).
110. Oxman,T., Arad,M., Klein,R., Avazov,N., & Rabinowitz,B. Limb ischemia preconditions the heart against reperfusion tachyarrhythmia. *Am J Physiol* **273**, H1707-H1712 (1997).
111. Kharbanda,R.K. *et al.* Transient limb ischemia induces remote ischemic preconditioning in vivo. *Circulation* **106**, 2881-2883 (2002).
112. Schmidt,M.R. *et al.* Intermittent peripheral tissue ischemia during coronary ischemia reduces myocardial infarction through a KATP-dependent mechanism: first demonstration of remote ischemic perconditioning. *Am J Physiol Heart Circ. Physiol* **292**, H1883-H1890 (2007).
113. Kerendi,F. *et al.* Remote postconditioning. Brief renal ischemia and reperfusion applied before coronary artery reperfusion reduces myocardial infarct size via endogenous activation of adenosine receptors. *Basic Res. Cardiol.* **100**, 404-412 (2005).

114. Andreka,G. *et al.* Remote ischaemic postconditioning protects the heart during acute myocardial infarction in pigs. *Heart* **93**, 749-752 (2007).
115. Basalay,M. *et al.* Remote ischaemic pre- and delayed postconditioning - similar degree of cardioprotection but distinct mechanisms. *Exp. Physiol* **97**, 908-917 (2012).
116. Gunaydin,B. *et al.* Does remote organ ischaemia trigger cardiac preconditioning during coronary artery surgery? *Pharmacol. Res.* **41**, 493-496 (2000).
117. Cheung,M.M. *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.* **47**, 2277-2282 (2006).
118. Hausenloy,D.J. *et al.* Effect of remote ischaemic preconditioning on myocardial injury in patients undergoing coronary artery bypass graft surgery: a randomised controlled trial. *Lancet* **370**, 575-579 (2007).
119. D'Ascenzo,F. *et al.* Cardiac remote ischaemic preconditioning reduces periprocedural myocardial infarction for patients undergoing percutaneous coronary interventions: a meta-analysis of randomised clinical trials. *EuroIntervention.* **9**, 1463-1471 (2014).
120. D'Ascenzo,F. *et al.* Remote ischaemic preconditioning in coronary artery bypass surgery: a meta-analysis. *Heart* **98**, 1267-1271 (2012).
121. Haji Mohd Yasin,N.A., Herbison,P., Saxena,P., Praporski,S., & Konstantinov,I.E. The role of remote ischemic preconditioning in organ protection after cardiac surgery: a meta-analysis. *J. Surg. Res.* **186**, 207-216 (2014).
122. Healy,D.A. *et al.* Remote preconditioning and major clinical complications following adult cardiovascular surgery: systematic review and meta-analysis. *Int. J. Cardiol.* **176**, 20-31 (2014).
123. McCrindle,B.W. *et al.* Remote ischemic preconditioning in children undergoing cardiac surgery with cardiopulmonary bypass: a single-center double-blinded randomized trial. *J. Am. Heart Assoc.* **3**, (2014).
124. Hong,D.M. *et al.* Does remote ischaemic preconditioning with postconditioning improve clinical outcomes of patients undergoing cardiac surgery? Remote Ischaemic Preconditioning with Postconditioning Outcome Trial. *Eur. Heart J.* **35**, 176-183 (2014).
125. Meybohm,P. *et al.* A Multicenter Trial of Remote Ischemic Preconditioning for Heart Surgery. *N. Engl. J. Med.*(2015).
126. Hausenloy,D.J. *et al.* Remote Ischemic Preconditioning and Outcomes of Cardiac Surgery. *N. Engl. J. Med.*(2015) **373**, 1408-17..
127. Pilcher,J.M. *et al.* A systematic review and meta-analysis of the cardioprotective effects of remote ischaemic preconditioning in open cardiac surgery. *J. R. Soc. Med.* **105**, 436-445 (2012).
128. Rahman,I.A. *et al.* Remote ischemic preconditioning in human coronary artery bypass surgery: from promise to disappointment? *Circulation* **122**, S53-S59 (2010).

129. Lucchinetti,E. *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* **116**, 296-310 (2012).
130. Kottenberg,E. *et al.* Protection by remote ischemic preconditioning during coronary artery bypass graft surgery with isoflurane but not propofol - a clinical trial. *Acta Anaesthesiol. Scand.* **56**, 30-38 (2012).
131. Thielmann,M. *et al.* Cardioprotective and prognostic effects of remote ischaemic preconditioning in patients undergoing coronary artery bypass surgery: a single-centre randomised, double-blind, controlled trial. *Lancet* **382**, 597-604 (2013).
132. Candilio,L. *et al.* Effect of remote ischaemic preconditioning on clinical outcomes in patients undergoing cardiac bypass surgery: a randomised controlled clinical trial. *Heart* **101**, 185-192 (2015).
133. Thygesen,K. *et al.* Third universal definition of myocardial infarction. *Circulation* **126**, 2020-2035 (2012).
134. Babu,G.G., Walker,J.M., Yellon,D.M., & Hausenloy,D.J. Peri-procedural myocardial injury during percutaneous coronary intervention: an important target for cardioprotection. *Eur Heart J* **32**, 23-31 (2011).
135. Iliodromitis,E.K. *et al.* Increased C reactive protein and cardiac enzyme levels after coronary stent implantation. Is there protection by remote ischaemic preconditioning? *Heart* **92**, 1821-1826 (2006).
136. Hoole,S. *et al.* Cardiac Remote Ischemic Preconditioning in Coronary Stenting (CRISP Stent) Study: a prospective, randomized control trial. *Circulation* **119**, 820-827 (2009).
137. Pei,H. *et al.* Remote ischemic preconditioning reduces perioperative cardiac and renal events in patients undergoing elective coronary intervention: a meta-analysis of 11 randomized trials. *PLoS. One.* **9**, e115500 (2014).
138. Botker,H.E. *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* **375**, 727-734 (2010).
139. Rentoukas,I. *et al.* Cardioprotective role of remote ischemic periconditioning in primary percutaneous coronary intervention: enhancement by opioid action. *JACC. Cardiovasc. Interv.* **3**, 49-55 (2010).
140. White,S.K. *et al.* Remote ischemic conditioning reduces myocardial infarct size and edema in patients with ST-segment elevation myocardial infarction. *JACC. Cardiovasc. Interv.* **8**, 178-188 (2015).
141. Crimi,G. *et al.* Remote ischemic post-conditioning of the lower limb during primary percutaneous coronary intervention safely reduces enzymatic infarct size in anterior myocardial infarction: a randomized controlled trial. *JACC. Cardiovasc. Interv.* **6**, 1055-1063 (2013).

142. Tamareille,S. *et al.* RISK and SAFE signaling pathway interactions in remote limb ischemic perconditioning in combination with local ischemic postconditioning. *Basic Res. Cardiol.* **106**, 1329-1339 (2011).
143. Eitel,I. *et al.* Cardioprotection by combined intrahospital remote ischaemic perconditioning and postconditioning in ST-elevation myocardial infarction: the randomized LIPSIA CONDITIONING trial. *Eur. Heart J.*(2015).
144. Wang,Z. *et al.* Confined ischemia may improve remote myocardial outcome after rat cardiac arrest. *Scand. J. Clin. Lab Invest* **74**, 27-36 (2014).
145. Wei,M. *et al.* Repeated remote ischemic postconditioning protects against adverse left ventricular remodeling and improves survival in a rat model of myocardial infarction. *Circ. Res.* **108**, 1220-1225 (2011).
146. Marongiu,E. & Crisafulli,A. Cardioprotection acquired through exercise: the role of ischemic preconditioning. *Curr. Cardiol. Rev.* **10**, 336-348 (2014).
147. Bolli,R. *et al.* Myocardial protection at a crossroads: the need for translation into clinical therapy. *Circ. Res.* **95**, 125-134 (2004).
148. Kloner,R.A. & Rezkalla,S.H. Cardiac protection during acute myocardial infarction: where do we stand in 2004? *J Am Coll. Cardiol.* **44**, 276-286 (2004).
149. Downey,J.M. & Cohen,M.V. Why do we still not have cardioprotective drugs? *Circ. J* **73**, 1171-1177 (2009).
150. Ludman,A.J., Yellon,D.M., & Hausenloy,D.J. Cardiac preconditioning for ischaemia: lost in translation. *Dis. Model. Mech.* **3**, 35-38 (2010).
151. Hausenloy,D.J. *et al.* Translating novel strategies for cardioprotection: the Hatter Workshop Recommendations. *Basic Res. Cardiol* **105**, 677-686 (2010).
152. Lecour,S. *et al.* ESC working group cellular biology of the heart: position paper: improving the preclinical assessment of novel cardioprotective therapies. *Cardiovasc. Res.* **104**, 399-411 (2014).
153. Kloner,R.A. & Schwartz,L.L. State of the Science of Cardioprotection: Challenges and Opportunities-- Proceedings of the 2010 NHLBI Workshop on Cardioprotection. *J Cardiovasc. Pharmacol. Ther.* **16**, 223-232 (2011).
154. Schwartz,L.L. *et al.* New horizons in cardioprotection: recommendations from the 2010 national heart, lung, and blood institute workshop. *Circulation* **124**, 1172-1179 (2011).
155. Araszkiewicz,A. *et al.* Postconditioning reduces enzymatic infarct size and improves microvascular reperfusion in patients with ST-segment elevation myocardial infarction. *Cardiology* **129**, 250-257 (2014).
156. Dwyer,N.B. *et al.* No cardioprotective benefit of ischemic postconditioning in patients with ST-segment elevation myocardial infarction. *J. Interv. Cardiol.* **26**, 482-490 (2013).
157. Kim,E.K. *et al.* Effect of ischemic postconditioning on myocardial salvage in patients undergoing primary percutaneous coronary intervention for ST-segment elevation myocardial infarction: cardiac magnetic resonance substudy of the POST randomized trial. *Int. J. Cardiovasc. Imaging* **31**, 629-637 (2015).

158. Kitakaze,M. *et al.* Human atrial natriuretic peptide and nicorandil as adjuncts to reperfusion treatment for acute myocardial infarction (J-WIND): two randomised trials. *Lancet* **370**, 1483-1493 (2007).
159. Lonborg,J. *et al.* Exenatide reduces reperfusion injury in patients with ST-segment elevation myocardial infarction. *Eur Heart J* **33**, 1491-1499 (2012).
160. Lonborg,J. *et al.* Exenatide reduces final infarct size in patients with ST-segment-elevation myocardial infarction and short-duration of ischemia. *Circ. Cardiovasc Interv.* **5**, 288-295 (2012).
161. Woo,J.S. *et al.* Cardioprotective effects of exenatide in patients with ST-segment-elevation myocardial infarction undergoing primary percutaneous coronary intervention: results of exenatide myocardial protection in revascularization study. *Arterioscler. Thromb. Vasc. Biol.* **33**, 2252-2260 (2013).
162. Bernink,F.J. *et al.* Effect of additional treatment with EXenatide in patients with an Acute Myocardial Infarction: the EXAMI study. *Int. J. Cardiol.* **167**, 289-290 (2013).
163. Ibanez,B. *et al.* Effect of Early Metoprolol on Infarct Size in ST-Segment-Elevation Myocardial Infarction Patients Undergoing Primary Percutaneous Coronary Intervention: The Effect of Metoprolol in Cardioprotection During an Acute Myocardial Infarction (METOCARD-CNIC) Trial. *Circulation* **128**, 1495-1503 (2013).
164. Pizarro,G. *et al.* Long-term benefit of early pre-reperfusion metoprolol administration in patients with acute myocardial infarction: results from the METOCARD-CNIC trial (Effect of Metoprolol in Cardioprotection During an Acute Myocardial Infarction). *J. Am. Coll. Cardiol.* **63**, 2356-2362 (2014).
165. Roolvink,V. *et al.* Rationale and design of a double-blind, multicenter, randomized, placebo-controlled clinical trial of early administration of intravenous beta-blockers in patients with ST-elevation myocardial infarction before primary percutaneous coronary intervention: EARLY beta-blocker administration before primary PCI in patients with ST-elevation myocardial infarction trial. *Am. Heart J* **168**, 661-666 (2014).
166. Thielmann,M. *et al.* Remote ischemic preconditioning reduces myocardial injury after coronary artery bypass surgery with crystalloid cardioplegic arrest. *Basic Res. Cardiol* **105**, 657-664 (2010).
167. Venugopal,V. *et al.* Remote ischaemic preconditioning reduces myocardial injury in patients undergoing cardiac surgery with cold-blood cardioplegia: a randomised controlled trial. *Heart* **95**, 1567-1571 (2009).
168. Wagner,R. *et al.* 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.* **11**, 758-762 (2010).
169. Karuppasamy,P. *et al.* Remote intermittent ischemia before coronary artery bypass graft surgery: a strategy to reduce injury and inflammation? *Basic Res. Cardiol.* **106**, 511-519 (2011).

170. Lucchinetti,E. *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* **116**, 296-310 (2012).
171. Young,P.J. *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* **107**, 1-10 (2012).
172. Iliodromitis,E.K. *et al.* Increased C- reactive protein and cardiac enzyme levels after coronary stent implantation. Is there protection by remote ischemic preconditioning? *Heart*(2006).
173. Ahmed,R.M. *et al.* Effect of remote ischemic preconditioning on serum troponin T level following elective percutaneous coronary intervention. *Catheter. Cardiovasc. Interv.* **82**, E647-E653 (2013).
174. Luo,S.J. *et al.* Remote ischemic preconditioning reduces myocardial injury in patients undergoing coronary stent implantation. *Can. J. Cardiol.* **29**, 1084-1089 (2013).
175. Davies,W.R. *et al.* Remote Ischemic Preconditioning Improves Outcome at 6 Years After Elective Percutaneous Coronary Intervention: The CRISP Stent Trial Long-term Follow-up. *Circ. Cardiovasc. Interv.* **6**, 246-251 (2013).
176. Zografos,T.A. *et al.* Effect of one-cycle remote ischemic preconditioning to reduce myocardial injury during percutaneous coronary intervention. *Am. J. Cardiol.* **113**, 2013-2017 (2014).
177. Liu,Z., Wang,Y.L., Hua,Q., Chu,Y.Y., & Ji,X.M. Late remote ischemic preconditioning provides benefit to patients undergoing elective percutaneous coronary intervention. *Cell Biochem. Biophys.* **70**, 437-442 (2014).
178. Prasad,A. *et al.* Remote ischemic preconditioning immediately before percutaneous coronary intervention does not impact myocardial necrosis, inflammatory response, and circulating endothelial progenitor cell counts: a single center randomized sham controlled trial. *Catheter. Cardiovasc. Interv.* **81**, 930-936 (2013).
179. Xu,X. *et al.* Effect of remote ischemic preconditioning in the elderly patients with coronary artery disease with diabetes mellitus undergoing elective drug-eluting stent implantation. *Angiology* **65**, 660-666 (2014).
180. Lavi,S. *et al.* Remote ischemic postconditioning during percutaneous coronary interventions: remote ischemic postconditioning-percutaneous coronary intervention randomized trial. *Circ. Cardiovasc. Interv.* **7**, 225-232 (2014).
181. Moretti,C. *et al.* The EUROpean and Chinese cardiac and renal Remote Ischemic Preconditioning Study (EURO-CRIPS): study design and methods. *J Cardiovasc. Med. (Hagerstown.)* **16**, 246-252 (2015).
182. Yellon,D.M. *et al.* Remote Ischemic Conditioning Reduces Myocardial Infarct Size in STEMI Patients Treated by Thrombolysis. *J. Am. Coll. Cardiol.* **65**, 2764-2765 (2015).

183. Sloth,A.D. *et al.* Improved long-term clinical outcomes in patients with ST-elevation myocardial infarction undergoing remote ischaemic conditioning as an adjunct to primary percutaneous coronary intervention. *Eur. Heart J.* **35**, 168-175 (2014).
184. Hausenloy,D.J. *et al.* Effect of remote ischaemic conditioning on clinical outcomes in patients presenting with an ST-segment elevation myocardial infarction undergoing primary percutaneous coronary intervention. *Eur. Heart J* **36**, 1846-1848 (2015).

Acknowledgements

We thank the British Heart Foundation (FS/10/039/28270) and the Rosetrees Trust for continued support. This work was supported by the National Institute for Health Research University College London Hospitals Biomedical Research Centre funding scheme, of which D.M.Y. is a senior investigator.

Author contributions

Both authors researched data for the article, and made substantial contributions to discussion of content, writing, reviewing, and editing the manuscript before submission.

Competing interests statement

The authors declare no competing interests.

Key points

Currently, no treatment has been proven to be effective for preventing ‘myocardial reperfusion injury’ — the death of cardiomyocytes that paradoxically occurs when reperfusing ischaemic myocardium

One or more brief cycles of ischaemia and reperfusion can protect the heart from acute myocardial infarction and myocardial reperfusion injury — a phenomenon termed ‘ischaemic conditioning’

Ischaemic conditioning can be applied either directly to the heart or from afar; that is, to a remote organ or tissue (such as an arm or a leg)

Investigation of signalling pathways underlying ischaemic conditioning has identified molecular targets for pharmacological manipulation — a therapeutic strategy termed ‘pharmacological cardioprotection’

Proof-of-concept clinical studies have shown mixed results of ischaemic conditioning in cardiac surgery and percutaneous coronary intervention; more consistently positive results have been observed in acute myocardial infarction

The results of large, multicentre, randomized, controlled clinical trials of ischaemic conditioning on clinical outcomes after cardiac surgery have highlighted the challenges in translating cardioprotection into clinical practice

Figure 1 | Ischaemic conditioning. This scheme depicts the different forms of ischaemic conditioning, and their timing with respect to the index myocardial ischaemia and reperfusion episode. The clinical settings in which they have been tested (black text) or the clinical settings in which there is potential for application (grey text) is described below. Delayed preconditioning with one or more brief episodes of ischaemia–reperfusion can be delivered 48–72 h before the index ischaemic event, whereas classical preconditioning has to be delivered within 3 h of the index ischaemic episode. Preconditioning can be delivered after the onset of index myocardial ischaemia, but

before reperfusion, whereas postconditioning has to be initiated within 1 min of reperfusion to be effective. Delayed postconditioning, which can be delivered up to 15–30 min into reperfusion has not yet been investigated in the clinical setting, and remains a preclinical observation.

Figure 2 | Signalling pathways of ischaemic conditioning. This scheme depicts the major signalling pathways and cardioprotective effects of the various forms of ischaemic conditioning. Remote ischaemic conditioning is performed by applying one or more cycles of brief ischaemia and reperfusion (IR) to the upper or lower limb by inflating and deflating a blood pressure cuff placed on the upper arm or thigh. Through the production of a blood-borne factor(s) and the stimulation of a neural pathway, the cardioprotective signal is conveyed to the heart where prosurvival signalling pathways within the cardiomyocyte mediate the cardioprotective effect. These signalling pathways are similar to those recruited by ischaemic preconditioning and postconditioning and targeted by pharmacological cardioprotection strategies. The signalling cascade underlying cardioprotection begins at the cardiomyocyte plasma membrane with the activation of G-protein-coupled or cytokine receptors by autacoids such as adenosine, bradykinin, or opioids (released in response to the ischaemic conditioning stimulus). This process results in the recruitment of signalling pathways such as the Reperfusion Injury Salvage Kinase (RISK) pathway (PI3K–Akt and MEK1/2–Erk1/2), Survivor Activator Factor Enhancement (SAFE) pathway (TNF and JAK–STAT), and the cGMP–PKG pathway. These salvage pathways have been shown to activate downstream mediators such as eNOS, GSK-3β, hexokinase II (HKII), PKC-ε, the mitochondrial ATP-

dependent potassium channel (K_{ATP}), which then mediate an inhibitory effect on mitochondrial permeability transition pore (MPTP) opening (adapted from[30]).

Table 1 | Major clinical studies of IPost in patients with STEMI

Study	*n*	Patient selection	IPost protocol	Main outcome	Notes
Positive studies					
Staat *et al*. (2005)[48]	30	LAD/RCA only ≤6 h ischaemic time TIMI 0 pre-PPCI TIMI 2–3 post-PPCI No collaterals No angina in 48 h	4 x 1 min inflations and deflations of angioplasty balloon upstream of stent Direct stenting	36% reduction in MI size (72 h AUC CK) Better blush grade	First clinical study to translate IPost into clinical setting
Ma *et al*. (2006)[50]	94	All STEMI ≤12 h ischaemic time TIMI 3 post-PPCI	3 x 0.5 min inflations and deflations of angioplasty balloon	27% and 32% reductions in MI size (peak CK and CK–MB) Better TIMI flow, WMSI, and endothelial function Less MDA	This study showed an alternative IPost protocol to be effective
Yang *et al*. (2007)[51]	41	All STEMI ≤12 h ischaemic time TIMI 0–1 pre-PPCI No collaterals	3 x 0.5 min inflations and deflations of angioplasty balloon	27% reduction in MI size (72 h AUC CK) 27% reduction in MI size (SPECT at 1 week)	First clinical study to demonstrate MI size reduction on SPECT
Thibault *et al.* (2008)[52]	38	LAD/RCA only ≤6 h ischaemic time TIMI 0 pre-PPCI TIMI 2–3 post-PPCI No collaterals No angina in 48 h	4 x 1 min inflations and deflations of angioplasty balloon upstream of stent Direct stenting	40% and 47% reductions in MI size (72 h AUC CK and troponin I) 39% reduction in MI size (SPECT at 6 months) 7% increase in LVEF (echo at 1 year)	First clinical study to demonstrate long-term benefit with IPost
Lonborg *et al.* (2010)[53]	118	All STEMI ≤12 h ischaemic time TIMI 0–1 pre-PPCI TIMI 3 post-PPCI	4 x 0.5 min inflations and deflations of angioplasty balloon within the stent	31% increase in myocardial salvage ratio 19% relative reduction in MI size (MRI at 3 months) 41% reduction in patients developing heart failure	First clinical study to demonstrate MI size reduction on MRI Largest positive study to date
Araszkiewicz *et al.* (2014)[155]	72	LAD/RCA/Cx-prox/mid ≤6 h ischaemic time TIMI 0 pre-PPCI No collaterals	4 x 1 min inflations and deflations of angioplasty balloon upstream of stent	36% reduction in MI size (36 h AUC CK) 26% reduction in MI size (36 h AUC CK–MB) Better blush grade Less MVO	Most recent positive study to date
Neutral or negative studies					
Sorensson *et al.* (2010)[54]	76	All STEMI ≤6 h ischaemic time TIMI 0 pre-PPCI	4 x 1 min inflations and deflations of angioplasty balloon within the stent	No difference in MI size (48 h AUC CK–MB, troponin T or MRI at day 6–9)	First neutral study, although reduced MI size in STEMI with large AAR (>30% LV)
Tarantini *et al.* (2012) POST-MI[56]	79	All STEMI <6 h ischaemic time TIMI 0–1 pre-PPCI No collaterals	4 x 1 min inflations and deflations of angioplasty balloon within the stent Direct stenting and no thrombectomy performed	No difference in MI size (MRI 30 days) — borderline increase	First study to suggest detrimental effects with IPost
Freixia *et al*. (2012)[55]	79	All STEMI <12 h ischaemic time TIMI 0–1 pre-PPCI No collaterals	4 x 1 min inflations and deflations of angioplasty balloon within the stent Direct stenting	No difference in MI size (MRI at 1 week or 6 months) Less myocardial salvage with IPost	Further study to suggest detrimental effects with IPost
Dwyer *et al.* (2013)[156]	102	All STEMI <6 h ischaemic time TIMI 0–1 pre-PPCI No collaterals	4 x 0.5 min inflations and deflations of angioplasty balloon at site of lesion	No difference in myocardial salvage or MI size (MRI at day 3)	First neutral study using an alternative IPost protocol
Hahn *et al*. (2014) POST[60]	700	All STEMI <12 h ischaemic time	4 x 1 min inflations and deflations of	No difference in ST-segment resolution,	Largest and first multicentre study

		TIMI 0–1 pre-PPCI	angioplasty balloon at site of lesion	myocardial blush grade, peak CK–MB levels or MACE (death, MI, severe heart failure, or stent thrombosis) No difference in MI size or myocardial salvage (MRI at day 3) in substudy of 111 patients[157]	
Eitel *et al.* (2015) LIPSIA CONDITIONING[143]	333	All STEMI	4 x 1 min inflations and deflations of angioplasty balloon at site of lesion vs control	No difference in MI size, myocardial salvage (MRI at day 3), or MACE at 6 months	Improved myocardial salvage when IPost combined with RIC
Ongoing studies					
DANAMI 3[67]	1,252	All STEMI <12 h ischaemic time TIMI 0–1 pre-PPCI	4 x 0.5 min inflations and deflations of angioplasty balloon at site of lesion	Primary outcome is all-cause death and heart failure at 2 years	Recruitment complete Currently in follow-up- results available early 2016

AAR, area at risk; AUC, area under curve; CK, creatine kinase; CK–MB, creatine kinase MB isoenzyme; echo, echocardiography; IPost, ischaemic postconditioning; LAD, left anterior descending artery; LV, left ventricle; LVEF, left ventricular ejection fraction; MACE, major adverse cardiac events; MI, myocardial infarction; PPCI, primary percutaneous coronary intervention; RCA, right coronary artery; RIC, remote ischaemic conditioning; SPECT, single-photon emission computed tomography; STEMI, ST-segment elevation myocardial infarction; TIMI, thrombosis in myocardial infarction; Cx-prox Proximal circumflex coronary artery; MDA, Malondialdehyde; MVO, microvascular obstruction; WMSI, wall motion score index.

Table 2 | Promising pharmacological strategies

Study	*n*	Patient selection	Treatment protocol	Main outcome	Notes
Natriuretic peptide					
Kitakaze *et al*. (2007) J-WIND[158]	569	All STEMI	Itravenous carperitide (atrial natriuretic peptide analogue) 72 h infusion before PPCI	15% reduction in MI size (72 h AUC total CK) 2.0% absolute increase in LVEF	Atrial natriuretic peptide targets prosurvival kinase pathways such as the cGMP and RISK pathways
Exenatide					
Lonborg *et al*. (2012)[159, 160]	107	All STEMI TIMI 0/1	Intravenous infusion of exenatide started 15 min before PPCI and continued for 6 h	23% reduction in MI size (3-month MRI) Increase in myocardial salvage index (0.62 to 0.71) Short ischaemic times (≤132 min) associated with greater myocardial salvage	Exenatide, a GLP-1 analogue, targets prosurvival kinase pathways such as the RISK pathway
Woo *et al.* (2013)[161]	58	All STEMI TIMI 0	Subcutaneous injection of exenatide before PPCI	52% reduction in MI size (1-month MRI) 27% reduction in MI size (72 h AUC CK–MB) 54% reduction in MI size (72 h AUC troponin I)	First study to demonstrate a positive effect with subcutaneously administered exenatide
EXAMI[162]	96	All STEMI TIMI 0/1	Intravenous infusion of exenatide started before PPCI and continued for 72 h	Ongoing study Primary end point will be MI size at 4 months as a percentage of AAR	Study completed, results awaited
EMPRES (NCT01938235)	198	All STEMI	Intravenous infusion of exenatide for 24 h (All-comer STEMI, TIMI 0/1)	Ongoing study Primary end point will be MI size at 3 months over AAR at 72 h after randomization (using MRI)	Largest clinical study to investigate exenatide
Metoprolol					
Ibanez *et al.* (2013) METOCARD-CNIC[163, 164]	270	LAD STEMI only	Intravenous metoprolol (3 x 5 mg) in ambulance before PPCI	22% reduction in MI size (7-day MRI) 3.7% absolute increase in LVEF (6-month MRI) 59% reduction in the incidence of poor LVEF (<35%; 6-month MRI) 65% reduction in need for ICD by 65% at 6 months 68% reduction in HHF at 2 years	The mechanism of cardioprotection is not clear
Roolvink *et al*. EARLY BAMI[165]	408	All STEMI <12 h after onset of symptoms	Intravenous metoprolol (3 x 5 mg) in ambulance before PPCI	Ongoing study Primary end point of MI size at 30 days on MRI	Largest study to investigate metoprolol

AAR, area at risk; AUC, area under curve; CK, creatine kinase; CK–MB, creatine kinase myocardial band; GLP-1, glucagon-like peptide 1; HHF, hospitalization for heart failure; ICD, implantable cardioverter–defibrillator; LAD, left anterior descending artery; LVEF, left ventricular ejection fraction; MI, myocardial infarction; PPCI, primary percutaneous coronary intervention; RISK, reperfusion injury salvage kinase; STEMI, ST-segment elevation myocardial infarction.

Table 3 | Major clinical studies of RIC in cardiac surgery

Study	Number of patients / type of surgery	Anaesthetic regimen	RIC protocol	Main outcome	Notes
Positive studies					
Cheung *et al*. (2006)[117]	37 children Corrective cardiac surgery Blood cardioplegia	Induction: sevoflurane Maintenance: fentanyl, isoflurane	4 x 5 min inflations/deflations of cuff on thigh vs control After anaesthesia and before surgical incision Sham: deflated cuff	Reduction in PMI (24 h troponin I AUC) Less inotrope requirement Lower airway pressures	First study in children to show benefit with RIC in cardiac surgery
Hausenloy *et al*. (2007)[118]	57 adults CABG surgery only ICCF or blood cardioplegia	Induction: etomidate, fentanyl, midazolam, pancuronium, propofol Maintenance: propofol	3 x 5 min inflations/deflations of cuff on upper arm vs control After anaesthesia and before surgical incision Sham: deflated cuff	43% reduction in PMI (72 h troponin T AUC)	First study in adult patients to show benefit with RIC in cardiac surgery
Thielmann *et al*. (2010)[166]	53 adults CABG surgery only Crystalloid cardioplegia	Induction: etomidate, rocuronium, sufentanil Maintenance: isoflurane or propofol	3 x 5 min inflations/deflations of cuff on upper arm vs control After anaesthesia and before surgical incision Sham: deflated cuff	45% reduction in PMI (72 h troponin I AUC)	No diabetic patients
Venugopal *et al*. (2007)[167]	57 adults CABG surgery with or without aortic valve surgery Blood cardioplegia	Induction: etomidate, fentanyl, midazolam, pancuronium, propofol Maintenance: propofol	3 x 5 min inflations/deflations of cuff on upper arm vs control After anaesthesia and before surgical incision Sham: deflated cuff	42% reduction in PMI (72 h troponin T AUC)	First study to demonstrate beneficial effects if RIC in presence of blood cardiopegia only
Wagner *et al*. (2010)[168]	81 adults CABG surgery with or without aortic valve surgery Crystalloid cardioplegia	Induction: diazepam, pancuronium, pufentanil Maintenance: diazepam, sufentanil	3 x 5 min inflations/deflations of cuff on upper arm vs control After anaesthesia and before surgical incision Sham: deflated cuff	12% reduction in PMI (8 h troponin I peak) Protective effect abolished by tramadol	First study to show modest effect with delayed RIC in cardiac surgery
Kottenberg *et al*. (2012)[130]	72 adults CABG surgery only Crystalloid cardioplegia	Induction: etomidate, sufentanil, rocuronium Maintenance: isoflurane–sufentanil or propofol–sufentanil	3 x 5 min inflations/deflations of cuff on upper arm vs control Four groups control propofol (n = 19), control isoflurane (n = 19), RIC propofol (n = 14), and RIC isoflurane (n = 19) Sham: deflated cuff	50% reduction in PMI (72 h troponin I AUC) Effect of RIC abolished in presence of propofol	First study to suggest that propofol might interfere with RIC protection No diabetic patients
Thielmann *et al*. (2013)[131]	198 adults CABG surgery only Crystalloid cardioplegia	Induction: etomidate, rocuronium, sufentanil Maintenance: isoflurane, sulfentanil	3 x 5 min inflations/deflations of cuff on upper arm vs control After anaesthesia and before surgical incision Sham: deflated cuff	17% reduction in PMI (72 h troponin I AUC) 73% reduction in all-cause mortality	First study to suggest limb RIC reducing mortality (secondary end point) No diabetic patients
Candilio *et al*. (2015)[132]	180 adults CABG surgery only Blood cardioplegia	Induction: etomidate, fentanyl, midazolam, pancuronium, propofol, rocuronium, vecuronium	2 x 5 min simultaneous inflations/deflations of cuffs on upper arm and thigh vs control After anaesthesia and before surgical incision	26% reduction in PMI (72 h high-sensitivity troponin T AUC) 54% reduction in postoperative AF	First clinical study to demonstrate beneficial effects with RIC on early

		Maintenance: fentanyl, isoflurane, propofol	Sham: deflated cuffs	1-day reduction in ITU stay	outcomes
Neutral studies					
Rahman *et al*. (2010)[128]	162 adults CABG surgery only Blood cardioplegia	Induction: etomidate, fentanyl, pancuronium Maintenance: alfentanil, propofol On CPB: enflurane or sevoflurane	3 x 5 min inflations/deflations of cuff on upper arm After anaesthesia and following surgical incision Sham: dummy arm	No differences in PMI (48 h AUC troponin T), arrhythmias, inotrope support, dialysis requirements, intubation times	First neutral study in CABG only patients
Kunst *et al*. (2011)[169]	54 adults CABG surgery with or without valve or aortic surgery Blood cardioplegia	Induction: atracurium, midazolam, remifentanil, propofol Maintenance: isoflurane On CPB: propofol	3 x 5 min inflations/deflations of cuff on upper arm After anaesthesia and before surgical incision Sham: deflated cuff	No differences in PMI (48 h AUC troponin I) or in release of inflammatory markers	First neutral study in CABG with or without valve surgery patients
Luchinetti *et al*. (2012)[170]	56 adults CABG surgery only Blood cardioplegia	Induction: fentanyl, propofol, remifentanil, rocuronium, sulfentanil Maintenance: isoflurane only	4 x 5 min inflations/deflations of cuff on thigh vs control After anaesthesia and before surgical incision Sham: none	No differences in PMI (48 h AUC high-sensitivity troponin T)	First study to suggest that RIC ineffective in presence of isoflurane, although propofol given at induction
Young *et al*. (2012)[171]	96 adults High-risk CABG surgery with or without valve, redo surgery	Induction: fentanyl, midazolam, rocuronium, vecuronium Maintenance: isoflurane, propofol	3 x 5 min inflations/deflations of cuff on arm vs control After anaesthesia and before surgical incision Sham: dummy arm	No differences in PMI (6 and 12 h levels of high-sensitivity troponin T) Possibility of increased PMI with RIC	First neutral study in high-risk cardiac surgery patients
Hong *et al*. (2014)[124]	1,280 adults CABG, valve, aortic, or congenial heart surgery Off-pump and on-pump	Induction: etomidate, midazolam, sufentanil Maintenance: propofol, remifentanil	4 x 5 min inflations/deflations of cuff on arm vs control Two RIC stimuli: one after anaesthesia before CPB or coronary anastomoses and a second immediately after the completion of CPB or coronary anastomoses Sham: none	No difference in primary combined end point of death, MI, arrhythmia, stroke, coma, renal failure or dysfunction, respiratory failure, cardiogenic shock, gastrointestinal complication, and multiorgan failure	**In this study, two RIC stimuli were tested**
Hausenloy *et al*. (2015) ERICCA[126]	1,612 adults CABG surgery with or without valve surgery Blood cardioplegia	Induction: etomidate, fentanyl, midazolam, pancuronium, propofol, rocuronium, vecuronium Maintenance: propofol, isoflurane	4 x 5 min inflations/deflations of cuff on upper arm vs control After anaesthesia and before surgical incision Sham: deflated cuff	No difference in primary combined end point (cardiac, death, MI, stroke, revascularization) No differences in PMI, AF, AKI, ITU/hospital stay, inotrope support, quality of life	Largest study to show no effect with RIC on one year outcomes
Meybohm *et al*. (2015) RIPHeart[125]	1,403 adults CABG surgery with or without valve surgery or aortic surgery Blood cardioplegia	Induction: propofol Maintenance: propofol	4 x 5 min inflations/deflations of cuff on upper arm After anaesthesia and following surgical incision Sham: dummy arm	No difference in primary combined end point (cardiac, death, MI, stroke, AKI) until hospital discharge No differences in	Largest study to show no effect with RIC on hospital outcomes

				ventilation time, ITU or hospital stay, AF, delirium	

AF, atrial fibrillation; AKI, acute kidney injury; AUC, area under curve; CPB, cardiopulmonary bypass; ITU, intensive therapy unit; MI, myocardial infarction; PMI, perioperative myocardial injury; RIC, remote ischaemic conditioning; ICCF, intermittent cross-clamp fibrillation.

Table 4 | Major clinical studies of limb RIC in planned PCI

Study	Number and condition of patients	RIC protocol	Main outcome	Comments
Positive studies				
Iliodromitis *et al.* (2006)[172]	41 Stable	3 x 5 min inflations/deflations of cuffs on both upper arms immediately before PCI Sham: deflated cuffs	Increase in 24 h levels and 48 h AUC of CK–MB (threefold to fourfold increase) and troponin I (threefold increase)	First study to test effect of limb RIC in planned PCI
Hoole *et al.* (2009) CRISP stent[136]	202 Stable	3 x 5 min inflations/deflations of cuff on upper arm immediately before PCI Sham: deflated cuff	57% reduction in troponin T at 24 h Less chest pain and fewer ischaemic electrocardiogram changes	First study to show cardioprotective effect with limb RIC in planned PCI
Ahmed *et al.* (2013)[173]	149 Stable	3 x 5 min inflations/deflations of cuff on upper arm immediately before PCI Sham: deflated cuff	57% reduction in troponin T at 16 h No difference in post-procedure MI	Second study to confirm benefits with RIC in this setting
Luo *et al.* (2013)[174]	205 Stable	3 x 5 min inflations/deflations of cuff on upper arm immediately before PCI Sham: deflated cuff	48% reduction in high-sensitivity troponin I at 16 h Reduced incidence of post-procedure (type 4a) MI (39% vs 54%)	First study to show positive effect of RIC on incidence of type 4a MI
Davies *et al.* (2013)[175]	192 Stable	3 x 5 min inflations/deflations of cuff on upper arm immediately before PCI Sham: deflated cuff	42% reduction in all-cause mortality, nonfatal MI, TIA or stroke, HHF at 6 years	First study to test effect of limb RIC on long-term clinical outcomes after PCI
Zografos *et al.* (2014)[176]	94 Stable	1 x 5 min inflations/deflations of cuff on upper arm immediately before PCI Sham: deflated cuff	80% reduction in troponin I at 24 h 56% reduction incidence of PCI-related MI	First study to show benefit with one cycle of limb RIC — beneficial in cases of *ad hoc* PCI
Liu *et al.* (2014)[177]	200 Stable	3 x 5 min inflations/deflations of cuff on upper arm immediately before PCI Sham: deflated cuff	40–60% reduction in troponin I and CK–MB at 24 h Less chest pain and ST-segment deviation with PCI	First study to test effect of second window of protection of limb RIC
Neutral or negative studies				
Iliodromitis *et al.* (2006)[172]	41 Stable	3 x 5 min inflations/deflations of cuffs on both upper arms immediately before PCI Sham: deflated cuffs	Increase in 24 h levels and 48 h AUC of CK–MB (threefold to fourfold increase) and troponin I (threefold increase)	First study to test effect of limb RIC in planned PCI
Prasad *et al.* (2013)[178]	95 Stable (75%) and unstable (25%)	3 x 3 min inflations/deflations of cuffs on upper arm immediately before PCI Sham: 3 x 3 min low-pressure inflations/deflations	No difference in the frequency of post-PCI myonecrosis, defined as a peak postprocedural cardiac troponin T level >0.03 ng/dl Increased levels of CK–MB at 24 h	Potential reasons for neutral results include older patients, more diabetics, suboptimal stimulus
Xu *et al.* (2014)[179]	200 Stable	3 x 5 min inflations/deflations of	No difference high-sensitivity troponin I levels at 16 h or incidence	First study to show neutral effect of RIC on incidence

	Aged ≥65 years and diabetic	cuff on upper arm immediately before PCI Sham: none	of post-PCI (type 4a) MI	of type 4a MI
Lavi *et al.* (2014)[180]	360 Stable (72%) and unstable (28%)	Three groups: 3 x 5 min inflations/deflations of cuffs on upper arm or thigh immediately after PCI vs sham Sham: 3 x 5 min low-pressure inflations/deflations	No difference in troponin T levels >3×URL post-PCI (at 6 h or 18–24 h) for either arm or leg RIC	First study to test effect of limb remote ischaemic postconditioning in planned PCI
Ongoing studies				
EURO-CRIPS[181]	555 Stable	3 x 5 min inflations/deflations of cuff on upper arm immediately before PCI	Planned study Primary end point will be contrast-induced acute kidney injury Secondary end point will be periprocedural myocardial injury	Largest clinical study to date

AUC, area under curve; CK-MB, creatine kinase MB isoenzyme; HHF, hospitalisation for heart failure; MI, myocardial infarction; PCI, percutaneous coronary intervention; RIC, remote ischaemic conditioning; TIA, transient ischaemic attack; URL, upper reference limit.

Table 5 | Major clinical studies of RIC in STEMI

Study	Number and population of patients	RIC protocol	Main outcomes	Notes
Bøtker *et al.* (2010) CONDI[138]	142 All STEMI	4 x 5 min inflations/deflations of cuff on upper arm in the ambulance before PPCI Sham: none	Increase in myocardial salvage index at 30 days No difference in MI size (SPECT or peak troponin)	First study to test effect of RIC in patients with STEMI Reduced MI size in LAD STEMI
Rentoukas *et al.* (2010)[139]	93 All STEMI	3 x 4 min inflations/deflations of cuff on upper arm at the hospital before PPCI Sham: 3 x 5 min low-pressure inflations/deflations	Better ST-segment resolution and lower peak troponin I Additive effects with morphine	Combined effects of RIC with morphine
Crimi *et al.* (2013)[141]	100 Anterior STEMI only	3 x 5 min inflations/deflation of cuff on thigh at onset of reperfusion Sham: none	20% reduction in 72 h AUC CK–MB **21**% reduction in myocardial oedema by MRI	First study to show effect of RIC given at onset of reperfusion, and first to report effect of RIC on enzymatic MI size and myocardial oedema
White *et al.* (2014) ERIC-STEMI[140]	83 All STEMI	4 x 5 min inflations/deflations of cuff on upper arm at the hospital before PPCI Sham: deflated cuff	27% reduction in MI size by MRI 19% reduction in myocardial oedema by MRI	First study to show effect of RIC given before PPCI on MI size and myocardial oedema by MRI
Hausenloy *et al.* (2015) ERIC-LYSIS[182]	519 All STEMI	4 x 5 min inflations/deflations of cuff on upper arm at the hospital before thrombolysis Sham: deflated cuff	17% reduction in enzymatic MI size (CK–MB and troponin T)	Only study to test effect of RIC in thrombolysed patients with STEMI
Sloth *et al.* (2014)[183]	251 All STEMI	4 x 5 min inflations/deflations of cuff on upper arm in the ambulance before PPCI Sham: none	51% reduction in all-cause mortality, nonfatal MI, TIA or stroke, HHF at 3.8 years	First study to test effect of RIC on long-term outcomes after PPCI (secondary end point)
Eitel *et al.* (2015) LIPSIA CONDITIONING[143]	333 All STEMI	4 x 5 min inflations/deflations of cuff on upper arm at the hospital before PPCI plus IPost Sham: none	Increased myocardial salvage with RIC + IPost vs control (49 vs 40) No difference in MI size, MVO, or 6-month clinical end points (death, re-infarction, and heart failure at 6 months)	Improved myocardial salvage when IPost combined with RIC Neither IPost alone nor RIC + IPost reduce myocardial oedema
CONDI-2/ERIC-PPCI[184]	4300 All STEMI	4 x 5 min inflations/deflations of cuff on upper arm before PPCI Sham: none or simulated	Ongoing study Primary end point of cardiac death and HHF at 12 months	Collaboration between Denmark, Serbia, Spain, and the UK First study to test effect of RIC on long-term clinical outcomes as primary end point

AUC, area under curve; CK-MB, creatine kinase MB isoenzyme; HHF, hospitalization for heart failure; IPost, ischaemic postconditioning; LAD, left anterior descending artery; MI, myocardial infarction; PPCI, primary percutaneous coronary intervention; RIC, remote ischaemic conditioning; SPECT, single-photon emission computed tomography; STEMI, ST-segment elevation myocardial infarction; TIA, transient ischaemic attack; MVO ,microvascular obstruction.

Author biographies

Derek J. Hausenloy is a Professor at the Hatter Cardiovascular Institute, University College London, and Professor in the Cardiovascular & Metabolic Disorders Program at Duke-National University Singapore. He is an Honorary Consultant Cardiologist at the Barts Heart Hospital and Senior Consultant at the National Heart Centre Singapore. He has published >147 papers, and his main research interests are cardioprotection and cardiac imaging in both the experimental and clinical settings.

Derek M. Yellon is Professor of Molecular & Cellular Cardiology at University College London, UK. He is director of the Hatter Cardiovascular Institute and head of the Research Department of Cardiovascular Medicine at UCL Hospitals & Medical School. He is also Programme Director (Cardiology & Diabetes) for the National Institute for Health Research–UCLH Biomedical Research Centre. He has published >480 papers and edited 23 books. He runs a translational research institute; his main research interests include myocardial protection, the pathophysiology of cardioprotection in setting of diabetes mellitus, ischaemia–reperfusion injury, molecular aspects of adaptation to ischaemic injury, and myocardial conditioning in both the basic and clinical arena.