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Novel targets and future strategies for acute cardioprotection: Position Paper of the European Society of Cardiology Working Group on Cellular Biology of the Heart

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Abstract

Ischaemic heart disease and the heart failure that often results, remain the leading causes of death and disability in Europe and worldwide. As such, in order to prevent heart failure and improve clinical outcomes in patients presenting with an acute ST-segment elevation myocardial infarction and patients undergoing coronary artery bypass graft surgery, novel therapies are required to protect the heart against the detrimental effects of acute ischaemia/reperfusion injury (IRI). During the last three decades, a wide variety of ischaemic conditioning strategies and pharmacological treatments

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have been tested in the clinic—however, their translation from experimental to clinical studies for improving patient outcomes has been both challenging and disappointing. Therefore, in this Position Paper of the European Society of Cardiology Working Group on Cellular Biology of the Heart, we critically analyse the current state of ischaemic conditioning in both the experimental and clinical settings, provide recommendations for improving its translation into the clinical setting, and highlight novel therapeutic targets and new treatment strategies for reducing acute myocardial IRI.

Keywords Cardioprotection • Ischaemia • Reperfusion • Myocardial Infarction • Ischaemic conditioning

1. The need for novel cardioprotective therapies

Although recent advances in treatment have improved survival in patients presenting with an acute myocardial infarction (AMI),[1] the number of patients going on to develop heart failure, a medical condition which exerts a huge global burden on healthcare and economic resources, has increased.[2,3] Despite timely reperfusion with primary percutaneous coronary intervention (PPCI), mortality and morbidity following ST-segment elevation myocardial infarction (STEMI) remain significant, with 7% death and 22% heart failure hospitalization at 1 year in patients presenting with an anterior STEMI.[4] For STEMI patients presenting with cardiogenic shock (about 10%), in-hospital mortality has been reported to be as high as 34%.[5] Furthermore, in developing countries, where ischaemic heart disease (IHD) is on the rise and treatment of AMI patients is not optimal, both mortality and morbidity rates also remain high.

Changes in patient demographics have meant that older and sicker patients with increasing co-morbidities [diabetes, left ventricular (LV) hypertrophy, renal failure] are undergoing coronary artery bypass graft (CABG) surgery, often with concomitant valve and/or aortic surgery, increasing the risk of peri-operative myocardial injury (PMI) and CABG-related myocardial infarction (MI) and worsening clinical outcomes.[6] A recent study from the UK reported a 28% rate of major adverse cardiac and cerebral events (MACCEs) at 1 year following CABG plus or minus valve surgery (cardiovascular death, non-fatal MI, coronary revascularization, and stroke at 12 months).[7]

As such, novel cardioprotective strategies are still required to attenuate the detrimental effects of acute myocardial ischaemia/reperfusion injury (IRI), so as to prevent adverse LV remodelling,[8] and reduce heart failure in patients with IHD. Interestingly, a recent UK cost-effectiveness analysis has demonstrated that a hypothetical cardioprotective agent capable of reducing MI size, preventing heart failure and reducing mortality in anterior STEMI patients treated by PPCI, would be very cost-effective.[9]

In this regard, the discovery, in 1986, that subjecting the heart to brief non-lethal cycles of ischaemia and reperfusion prior to a lethal episode of acute IRI dramatically reduced MI size, a phenomenon termed 'ischaemic pre-conditioning' (IPC),[10] has provided a powerful endogenous strategy for cardioprotection. It has evolved from IPC (classical and delayed, both of which are limited in their clinical application as they are invasive and need to be applied prior to ischaemia),[10–12] to ischaemic post-conditioning (IPost)[13,14] (which allows the intervention to be applied at the time of reperfusion, but is still invasive), to remote ischaemic conditioning (RIC)[15] (which has allowed the intervention to be applied non-invasively to the arm or leg, even during ongoing myocardial ischaemia and at reperfusion), making it more clinically applicable.

Although 30 years of research on ischaemic conditioning have provided important insights into the complex intracellular signalling pathways underlying cytoprotection at the level of the cardiomyocyte, the translation of ischaemic conditioning into the clinical setting for patient benefit has been largely disappointing. A vast number of cardioprotective therapies for reducing MI size in the laboratory setting have failed to demonstrate any benefit in the clinical setting; and even for the therapies which have been shown to reduce MI size in STEMI patients or reduce PMI in CABG patients, successful demonstration of improved clinical outcomes has been elusive.[16–21] At this juncture, it is important to assess what we have learned after 30 years of research on ischaemic conditioning and what we can do to improve its translation into the clinical setting for patient benefit. *Figure 1* provides an overview of the current state of ischaemic conditioning.

Therefore, in this Position Paper of the European Society of Cardiology Working Group on Cellular Biology of the Heart, we critically analyse the current state of ischaemic conditioning in both the experimental and clinical settings, provide recommendations for improving the translation of novel cardioprotective therapies into the clinical setting, and highlight novel therapeutic targets and new treatment strategies for reducing acute myocardial IRI and improving clinical outcomes in patients with IHD. In this Position Paper, the focus will be on acute cardioprotective strategies targeting myocardial IRI, rather than primary prevention strategies, and those therapies directed to preventing adverse post-MI remodelling.

The current Position Paper will focus on a number of important recent developments in the field of cardioprotection, which have taken place in the last 2–3 years, since the publication of our previous two Position Papers providing recommendations on optimizing pre-clinical and clinical cardioprotecton studies.[18,19] Several neutral large scale clinical outcomes studies in cardioprotection[4,7,22,23] and a number of neutral proof-of-concept clinical cardioprotection studies in STEMI patients have been recently published and will be discussed in the current Position Paper. In addition, several novel targets and new strategies for cardioprotection have emerged over the last 2–3 years and are highlighted in this Position Paper.

2. Why have there been so many recent neutral clinical cardioprotection studies?

In the last few years, there have been an increasing number of neutral clinical cardioprotection studies in both STEMI (*Table 1*) and CABG patients. The reasons for the neutral outcomes are varied and have been extensively reviewed and discussed in the recent literature,[17,18,20,21,24] and only an overview is provided here

2.1. Endogenous cardioprotection strategies

2.1.1 Adenosine

Both experimental and clinical studies of AMI with adenosine administered at the time of reperfusion have had mixed results in terms of reducing MI size, with *post-hoc* analyses suggesting beneficial effects in STEMI patients presenting within 3 h of symptom onset.[25–29]

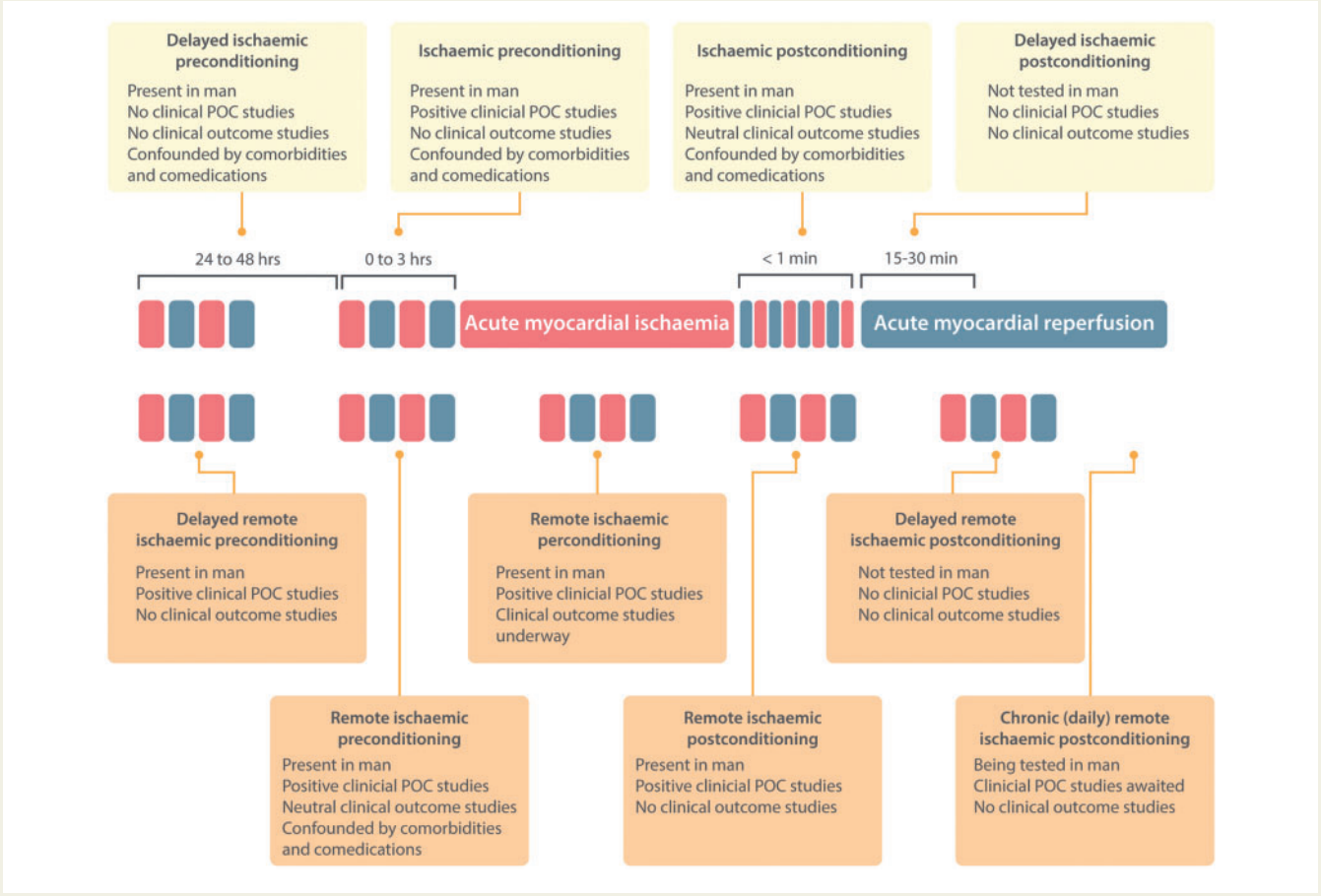

Figure 1 The current state of ischaemic conditioning. This figure provides an overview of the various forms of ischaemic conditioning and their current states in terms of their translation into the clinical setting. So far, none of these have been implemented as clinical therapy. Cardioprotection can be elicited by applying brief cycles of ischaemia and reperfusion directly to the heart either: (i) 24–48 h prior the myocardial index ischaemia (delayed ischaemic pre-conditioning); (ii) within 3 h of the index myocardial ischaemia (IPC); (iii) within 1 min of reperfusion following the index myocardial ischaemia (IPost); and (iv) 15–30 min after the onset of myocardial reperfusion following the index myocardial ischaemia (delayed ischaemic post-conditioning). Cardioprotection can also be induced by applying brief cycles of ischaemia and reperfusion to an organ or tissue (such as the arm or leg) away from the heart either: (i) 24–48 h prior the index myocardial ischaemia (delayed remote ischaemic pre-conditioning); (ii) within 3 h of the index myocardial ischaemia (remote IPC); (iii) during the index myocardial ischaemia (remote ischaemic perconditioning); (iv) within 1 min of reperfusion following the index myocardial ischaemia (remote IPost); and (v) 15–30 min after the onset of myocardial reperfusion following the index myocardial ischaemia (delayed remote IPost); and (vi) on a daily basis for 1 month (chronic RIC). (POC, proof of concept).

Interestingly, a meta-analysis of clinical studies undertaken in the PPCI era has demonstrated a beneficial effect of intracoronary adenosine in terms of less heart failure following STEMI.[30]

In summary, the results with adenosine have had mixed results in proof-of-concept clinical cardioprotection studies, but it appears that STEMI patients presenting with short ischaemic times or those receiving intracoronary adenosine, may be more likely to benefit.

2.1.2 Atrial natriuretic peptide

Experimental studies have reported cardioprotection with atrial natriuretic peptide (ANP) administered at the time of reperfusion,[31] and a clinical study has demonstrated a modest (15%) reduction in MI size (measured by total serum creatine kinase) with an infusion of carperitide (an ANP agonist) initiated prior to PPCI in STEMI patients.[32]

Therefore, ANP has shown promise as a therapy for reducing MI size, but whether it can improve clinical outcomes is not known and needs to be determined.

2.1.3 Exenatide- a GLP-1 analogue

Exenatide is a synthetic version of the glucagon-like-peptide-1 (GLP-1) analogue, exendin-4, a peptide derived from a lizard venom, which has been reported to reduce MI size when administered prior to reperfusion in small and large animal MI models.[33–35] Two small proof-of-concept clinical studies in STEMI patients have reported beneficial effects with either intravenous or subcutaneous exenatide initiated prior to PPCI.[36,37] Most benefit was observed in those STEMI patients presenting within 132 min of symptom onset,[38] although exenatide was found to not improve long-term clinical outcomes in this group of patients.[39] A recent study by Roos *et al.*[40] failed to find any beneficial effect of IV exenatide on MI size normalized for area-at-risk (AAR). The ongoing Exenatide for Myocardial Protection During

Table 1 Major recent cardioprotection studies in STEMI patients which have had neutral results

Study	Treatment strategy	Main findings	Experimental and clinical data	Patient population	Timing of treatment	Why the clinical study may have failed to show cardioprotection
Lincoff *et al.* (2014) PROTECT-MI[214]	Delcasertib PKC-δ inhibitor which prevents apoptotic cell death	1010 patient study with no effect of IV infusion of Delcasertib at 3 different doses on acute MI size (AUC CK-MB)	Positive small and large animal data. Inconsistent cardioprotection in experimental studies[215] One positive small proof-of-concept clinical study[216]	Ischaemic time ≤6 h Large AAR Included TIMI > 1	2.5 h infusion initiated prior to PPCI	Single targeted approach Inconsistent cardioprotection in experimental studies Drug given IV although initial POC study used IC route Patient population not optimized
Erlinge *et al.* (2014) CHILL-MI[217]	Therapeutic hypothermia	120 patient study with no effect of therapeutic hypothermia on acute MI size (by CMR 2–6 days)	Positive small and large animal data but not protective at reperfusion[203] One positive small proof-of-concept clinical study[218]	Ischaemic time ≤ 6 h Small and large AAR Included TIMI > 1	Therapeutic hypothermia for 1 h initiated prior to PPCI (delay in PPCI by 9 min)	Experimental data showed not protective at reperfusion Patient population not optimized
Siddiqi *et al.* (2014) NIAMI[211]	Nitrite NO donor targeting cGMP/PKG cardioprotective pathway	229 patient study with no effect of IV nitrite (70 μmol) on acute MI size (by CMR 6–8 days)	Positive small and large animal data, but neutral in NIH CESAR multicentre testing[210]	Ischaemic time <12 h Small and large AAR TIMI ≤ 1	5 min bolus prior to PPCI	Single targeted approach Inconsistent cardioprotection in experimental studies Patient population not optimized Dose not optimized >90% of patients received GTN prior to IV nitrites
Jones *et al.* (2015)[212]	Nitrite NO donor targeting cGMP/PKG cardioprotective pathway	80 patient study with no effect of IC nitrite (1.8 μmol) on acute MI size (by total CK) In patients with TIMI < 1 there was a reduction in MI size	Positive small and large animal data, but neutral in NIH CESAR multicentre testing[210]	Ischaemic time ≤6 h Small and large AAR Included TIMI > 1	Nitrite bolus given after crossing lesion with guidewire	Single targeted approach Inconsistent cardioprotection in experimental studies Patient population not optimized Dose not optimized
Atar *et al* 2015 MITOCARE[219]	TRO40303 Mitochondrial agent targeting translocator protein	163 patient study with no effect of IV TRO40303 on acute MI size (by 72 h AUC CK and TnI)	Positive small animal studies only[220]	Ischaemic time ≤ 6 h Small and large AAR TIMI ≤ 1	TRO40303 bolus Prior to PPCI	Single targeted approach Dose in clinical study lower than experimental studies Patient population not optimized

Continued

Table 1 *Continued*

Study	Treatment strategy	Main findings	Experimental and clinical data	Patient population	Timing of treatment	Why the clinical study may have failed to show cardioprotection
						Dose not optimized Higher rate of cardiac events in the TRO40303 group.
Gibson *et al* 2015 EMBRACE STEMI[221]	MTP-131 Mitochondrial peptide targeting cardiolipin	118 patient study with no effect of IV MTP-131 infusion on acute MI size (by 72 h AUC CK-MB)	Positive small and large animal studies[222]	Ischaemic time ≤4 h Large AAR TIMI ≤ 1	MTP-131 infusion initiated Prior to PPCI	Single targeted approach Dose not optimized
Cung *et al* 2015 CIRCUS[4]	Cyclosporin-A Mitochondrial PTP inhibitor	970 patients study with no effect of IV cyclosporine-A on one year clinical endpoints (death, heart failure, and adverse LV remodelling)	Positive small and large animal studies Inconsistent cardioprotection in experimental studies[223–225] One positive small proof-of-concept clinical study[71]	Ischaemic time < 12 h Large AAR TIMI ≤ 1 No collaterals	CsA bolus prior to PPCI	Single targeted approach Inconsistent cardioprotection in experimental studies[223,225] Not effective in co-morbidity animal model Dose not optimized Patient population partially optimized Greater use of P2Y12 platelet inhibitors (prasugrel, ticagrelor) which are known to reduce MI size *per se*[159]
Latini *et al* 2016 CYCLE[226]	Cyclosporin-A Mitochondrial PTP inhibitor	410 patients study with no effect of IV cyclosporine-A on ST-segment resolution	Positive small and large animal studies Inconsistent cardioprotection in experimental studies[223–225] One positive small proof-of-concept clinical study[71]	Ischaemic time ≤6 h Small and large AAR TIMI ≤ 1	CsA bolus 5 min prior to PPCI	Single targeted approach Inconsistent cardioprotection in experimental studies Dose not optimized Patient population not optimized Greater use of P2Y12 platelet inhibitors (prasugrel, ticagrelor) which are known to reduce MI size *per se*[159]
Janssen *et al.* (2015) NOMI (NCT01398384)	Inhaled NO (vasoKINOX 450)	250 patients study with no effect of inhaled NO	No animal data with inhaled NO	Ischaemic time <12 h Small and large AAR	Inhaled NO for 4 h initiated prior to PPCI	Single targeted approach Lack of experimental data

Continued

Table 1 *Continued*

Study	Treatment strategy	Main findings	Experimental and clinical data	Patient population	Timing of treatment	Why the clinical study may have failed to show cardioprotection
	Targets cGMP/PKG cardioprotective pathway	on acute MI size (by CMR day 3)		Included TIMI > 1 Collaterals not excluded		Dose not optimized Patient population not optimized Prior use of GTN may have interfered with cardioprotection as reduction in MI size observed in those patients who had not received GTN in the ambulance
Engstrom *et al.* (2016) DANAMI-3 IPOST[46]	IPost	617 patients study with no effect of IPost (4 × 30 s) on 38-month clinical endpoints (death, heart failure)	Positive small and large animal studies[14,227,228] Inconsistent cardioprotection in clinical studies[44,45]	Ischaemic time <12 h Small and large AAR Included TIMI > 1	At time of reperfusion	Patient population not optimized Inconsistent cardioprotection in previous clinical studies IPost protocol not optimized Study underpowered to detect improvement in clinical outcomes.
Roolvink *et al.* (2016) Early BAMI[69]	Metoprolol Reduces myocardial oxygen consumption	342 patients study with no effect of IV metoprolol (2 × 5 mg) on MI size on CMR at 30 days	One positive large animal study[66] One positive proof-of-concept clinical study[67]	Ischaemic time <12 h Small and large AAR Included TIMI > 1	At time of reperfusion	Patient population not optimized Therapy more effective when given in ambulance Dose used less than that used in prior positive study[67]
Roos *et al.* (2016) EXAMI[40]	Exenatide GLP-1 analogue which activates pro-survival signalling pathways	91 patients study with no effect of IV exenatide on MI size on CMR at 1 month over AAR acutely (T2 CMR)	Positive small and large animal studies[33,34] Two previous positive clinical studies[36,37]	TIMI ≤ 1	Prior to reperfusion	Patient population not optimized Dose used different from prior positive studies[36,37]
Verouhis *et al.* (2016) RECOND[61]	RIC	93 patient study with no effect of lower limb RIC (variable cycles up	Positive small and large animal studies[229]	Ischaemic time <6 h Large AAR Included TIMI > 1	At least one RIC cycle prior to reperfusion	Patient population not optimized

Continued

Table 1 *Continued*

Study	Treatment strategy	Main findings	Experimental and clinical data	Patient population	Timing of treatment	Why the clinical study may have failed to show cardioprotection
		to 7 until PPCI completed) on myocardial salvage index (day 4–7 CMR)	Six previous positive clinical studies[54–59]			Variable number of RIC cycles used whereas most positive clinical studies only gave four cycles

Reperfusion Study is also testing the effect of IV exenatide on final MI size at 3 months over AAR at 72 h post-randomization (assessed by CMR).

In summary, the results with exenatide have had mixed results in proof-of-concept clinical cardioprotection studies, in part due to the variable doses tested in each trial. As such, further studies are required to determine the optimum cardioprotective dose prior to undertaking clinical outcome studies.

2.1.4 Ischaemic post-conditioning

Following the first positive clinical study showing a reduction in MI size with IPost (4 × 1 min cycles of alternate angioplasty balloon inflation/deflation),[41] the results of subsequent clinical studies have been mixed.[42–45] The reasons for this are unclear, but probably relate to patient selection and the IPost protocol itself (durations of inflations/deflations, site of IPost in stent or upstream of stent).[21] The DANAMI-3 IPost study,[46] which tested the effect of IPost (3- × 30-s cycles of alternate angioplasty balloon inflation/deflation) on long-term clinical outcomes, found a non-significant reduction in major adverse cardiac events (all cause death and heart failure hospitalization at 38 months), but this study was probably underpowered to detect this endpoint, given the low event rate in this STEMI population.

In summary, the results with IPost have had mixed results in proof-of-concept clinical cardioprotection studies. Whether IPost can improve clinical outcomes remains unclear and needs to be tested in a suitably powered large multi-centre randomized clinical trial.

2.1.5 Remote ischaemic conditioning

RIC, using one or more cycles of brief limb ischaemia and reperfusion, has been found in both small and large animal MI models to reduce MI size.[47–53] At least seven clinical studies have shown RIC to reduce acute MI size or increase myocardial salvage in STEMI patients treated by PPCI, when assessed by serum cardiac enzymes, SPECT, and CMR.[54–60] However, there has been one recently published neutral clinical study by Verouhis *et al.* (2016) (RECOND trial),[61] in which limb RIC (up to seven cycles of lower limb RIC) with at least one cycle initiated prior to reperfusion failed to reduce MI size as a percentage of the AAR (assessed by CMR at 4–7 days) in 93 anterior STEMI patients. Why this study was neutral is not clear but it may relate to the variable and high number of RIC cycles used, and the prior treatment with ticagrelor and clopidogrel in a large number of patients.[61]

Whether RIC can improve clinical outcomes is currently unknown, although it has been shown that STEMI patients undergoing RIC in the ambulance during transportation to PPCI had reduced MACCEs and all-cause mortality within 4 years after the index event,[62] and lowered economical expense of medical resources of hospitalization for post-infarction heart failure.[63] However, these studies were not powered for clinical outcome analyses.[64] The results of the ongoing CONDI-2/ERIC-PPCI, which will investigate the effect of RIC on cardiac death and hospitalization for heart failure at one year in reperfused STEMI patients, are eagerly awaited.[65]

In summary, limb RIC is the only therapy which has shown largely positive data in proof-of-concept clinical cardioprotection studies, and the CONDI-2/ERIC-PPCI trial will determine whether this non-invasive, low-cost intervention, can improve clinical outcomes in reperfused STEMI patients.

2.2. Beta-blocker therapy

2.2.1 Metoprolol

Data from a large-animal MI model found that intravenous administration of the β1-selective blocker, metoprolol, prior to reperfusion, reduced MI size.[66] In the 270 anterior STEMI patient METOCARD-CNIC trial, intravenous metoprolol (3 × 5 mg) administered in the ambulance prior to PPCI reduced MI size, prevented LV adverse remodelling, preserved LV systolic function, and lowered hospital re-admissions for heart failure.[67,68] Unfortunately, the EARLY BAMI trial failed to report a reduction in MI size at 1 month (assessed by CMR) with IV metoprolol (2 × 5 mg) administered prior to PPCI in STEMI patients presenting within 12 h of symptom onset.[69] The reasons for the neutral results of the EARLY BAMI trial vs. the METOCARD-CNIC trial include: dosing (10 vs. 15 mg), timing (most benefit observed with metoprolol given soon after STEMI onset), patient population (all-comers vs. anterior STEMI), and endpoint assessment (1 month vs. first week—CMR performed in the first week following PPCI may over-estimate MI size unless long intervals between gadolinium salt injection and image acquisition are used[70]). Therefore, this therapeutic approach may not be suitable for all STEMI patients, and those with heart failure, hypotension or presenting with AV-block will not qualify for this therapy. Whether this therapeutic approach can improve clinical outcomes in reperfused STEMI patients will be addressed by the MOVE ON! randomized clinical trial, which will investigate the effect of metoprolol on cardiac death and heart failure hospitalization.

In summary, the results with metoprolol have had mixed results in proof-of-concept clinical cardioprotection studies, in part due to the patient selection and the timing and dose used. As such, further studies are required to determine the optimum cardioprotective dose prior to undertaking clinical outcome studies.

2.3. Mitochondria-targeted cardioprotection strategies

2.3.1 Cyclosporine-A

A proof-of-concept clinical study demonstrated a reduction in MI size and less adverse LV remodelling with an IV bolus of Cyclosporine-A (CsA, 2.5 mg/kg Sandiummune), administered prior to reperfusion, in 58 reperfused STEMI patients (<12 h of symptoms and pre-PPCI TIMI flow <1).[71,72] However, one small clinical study in thrombolysed STEMI patients,[73] and two subsequent large multicentre randomized clinical trials have failed to demonstrate a reduction in MI size or improved clinical outcomes with CsA administered prior to PPCI in STEMI patients.[4,23] In the CIRCUS trial, an IV bolus of CsA (2.5 mg/kg Ciclomulsion) administered prior to reperfusion failed to reduce MI size and improve 1 year clinical outcomes (death, heart failure hospitalization and adverse LV remodelling) in 791 STEMI patients, when compared with placebo. Furthermore, in the CYCLE trial, an IV bolus of CsA (2.5 mg/kg Sandimmune) administered prior to reperfusion, failed to improve ST-segment resolution and reduce MI size in 410 STEMI patients.[23] Why these large clinical studies were neutral is not clear, but it may have been due to an inadequate dose and a changing patient population (increased use of P2Y12 platelet inhibitors).[74,75] The fact that studies in large animal hearts by Jennings' group[76,77] have shown that few cardiomyocytes can be salvaged by reperfusion in the canine heart after 3 h and none after 6 h of ischaemia have passed suggests that patients receiving 6–12 h of ischaemia may not respond to therapies applied at the time of reperfusion.

In summary, the results with CsA have been largely neutral, and this may have been due to patient selection and the dose of CsA. As such, mitochondrial permeability transition pore (PTP) inhibition with more potent and selective agents is required to investigate whether this therapeutic strategy is effective in reperfused STEMI patients.

2.4. Clinical cardioprotection studies in CABG patients

In this section, we review the major factors which may have contributed to the neutral results of recent clinical cardioprotection studies in CABG patients and propose strategies for optimizing the design of future clinical studies, in order to improve the translation of cardioprotection into the clinical setting. Many of the factors relevant to STEMI patients also apply to clinical studies in CABG patients and may have contributed to the neutral results in these studies.

In CABG surgery the magnitude of acute myocardial IRI and infarction is much less than that which occurs in reperfused STEMI patients, which may make it more difficult to demonstrate a beneficial effect with a novel cardioprotective strategy. In addition, the aetiology of PMI following CABG not only includes acute IRI, but also other factors such as directly handling of the heart, inflammation, and coronary microembolization, and these may not have been amenable to ischaemic conditioning.[6] Furthermore, the majority of clinical studies have investigated novel therapies, which were tested in animal models of AMI and which are closer in design to the STEMI than the CABG setting. Therefore, therapies which are intended to be investigated in the CABG setting should ideally be tested using animal models of cardiopulmonary bypass surgery.[19]

Confounding effects of co-medication given to CABG patients, such as propofol and opioids, may have contributed to the neutral results of the ERICCA and RIPHeart studies, which failed to demonstrate any beneficial effects of RIC on clinical outcomes in patients undergoing CABG surgery.[7,22,78] Other drugs given to patients undergoing CABG surgery, which may interfere with cardioprotection include nitrates, beta blockers, inhaled anaesthetics (such as isoflurane) and so on.[79–81] Therefore, experimental studies should investigate whether future therapies can protect against acute myocardial IRI in the presence of co-medication used during CABG surgery.

3. Novel therapeutic targets for cardioprotection

Targeting standard signalling pathways underlying ischaemic conditioning has not been successful. As such there is a need to discover and investigate novel therapeutic targets for cardioprotection (see *Figure 2* for overview). Over the past 30 years of research in this area, enthusiasm for some particular cardioprotective strategies such as cariporide, erythropoietin, oxygen free radical scavengers or calcium entry blockers has waned, even if trial design may have accounted for some of the disappointing outcomes.[16–21,52] In the case of GIK, the situation may be changing as the only clinical study in which it was administered systematically before PPCI (in the ambulance) was positive in STEMI patients.[82] However, other targets have undergone a renaissance as new aspects are discovered. For example, despite disappointing clinical trials of ROS scavengers, there is renewed optimism for a more targeted approach directed to preventing mitochondrial ROS production at the time of reperfusion.[83–85] Nitric oxide (NO) is fundamental to many protective strategies, and although NO donors and nitrites have produced disappointing results in the clinical

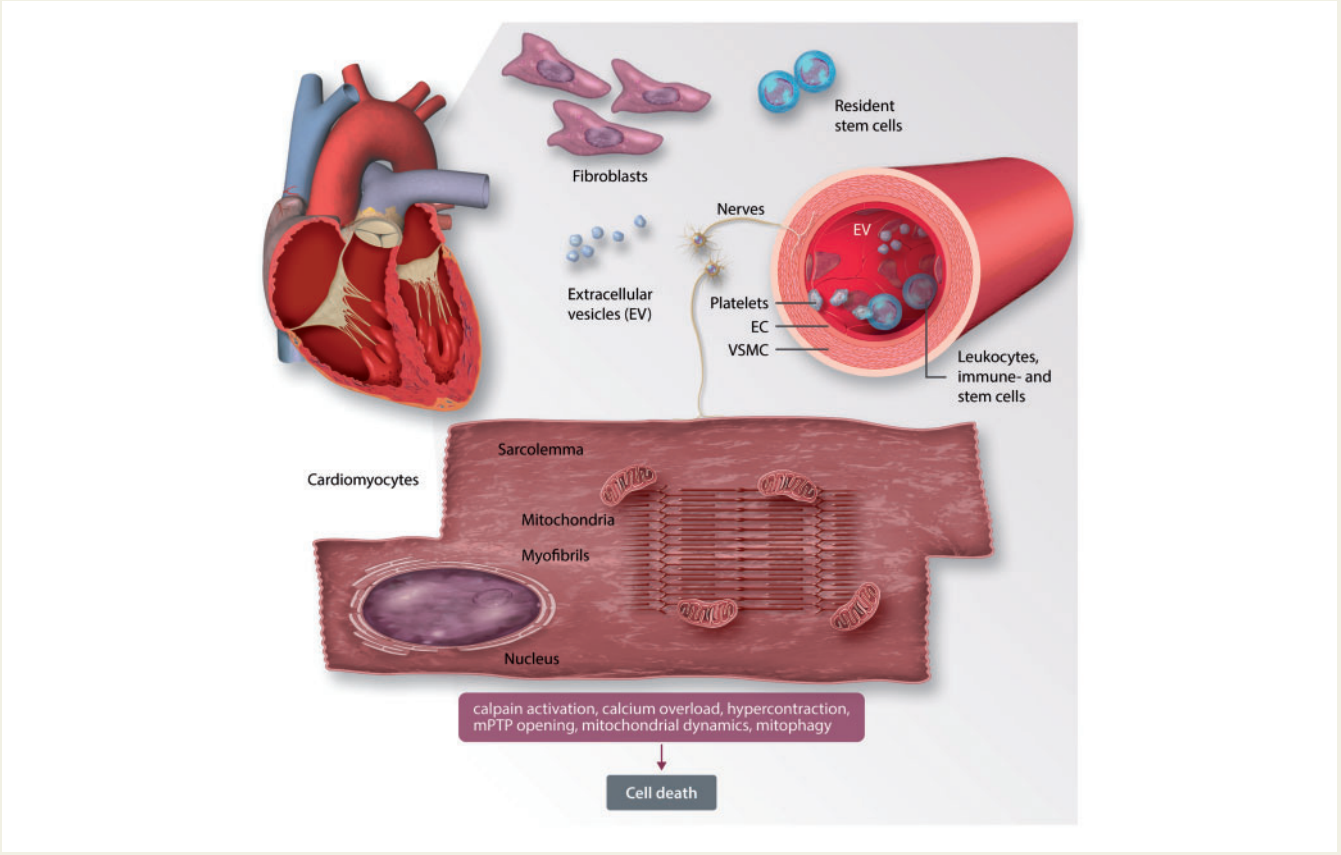

Figure 2 Myocardial IRI affects many cell types which then signal to cardiomyocytes. Cardiomyocyte injury occurs at the level of the sarcolemma, myofibrils, SR, mitochondria, and the nucleus. EC, endothelial cells, VSMC, vascular smooth muscle cells.

setting, optimism remains for approaches that manipulate tetrahydrobiopterin and particulate or soluble guanylate cyclase.[86]

Initial trials of broad anti-inflammatory agents have been disappointing, perhaps unsurprisingly, given what we now know about its Jekyll-and-Hyde nature.[87] New evidence suggests potential roles for neutrophils and platelets.[87,88] The discovery of novel regulatory mechanisms such as lncRNA and miRNA has presented new opportunities,[89] although a causal role for miRNA in cardioprotection is still controversial.[90,91]

To date, most cardioprotective strategies have either been designed to target and inhibit a crucial cell death pathway, or to activate a specific endogenous cardioprotective pathway. The major mechanism of cell death occurring rapidly after reperfusion is necrosis, as demonstrated by tetrazolium staining of animal hearts or cardiac biomarker release in clinical studies. The role of apoptosis is less clear. Although it may be involved in infarct expansion, the evidence for its involvement in early reperfusion injury is controversial.[92–95] A recent experimental study has shown that cardiac-specific deletion of caspase 3 and 7 had no impact on MI size and subsequent LV remodelling, indicating no role of apoptosis in IRI.[95] MI size can also be significantly reduced by inhibitors of necroptosis[96,97] or pyroptosis,[51] implicating these forms of cell death and their underlying mechanisms as potential targets. Autophagy is also involved, although it may play opposing roles during ischaemia and reperfusion.[98] Matrix metalloproteinase-2 (MMP2) inhibition by ischaemic conditioning or MMP inhibitors has been demonstrated to reduce MI size in experimental studies, even in the presence of hypercholesterolaemia, and MMP seems to be a promising biomarker for the development of IHD.[99–101]

In terms of activating cardioprotective pathways, there is an abundance of literature demonstrating cardioprotection in cell or animal models by receptor ligands that activate the reperfusion injury salvage kinase (RISK) or survival activating factor enhancement (SAFE) pathways.[102–104] However, novel pathways or combinations of pathways should also be considered. For example, PKG has been validated as a target for cardioprotection in humans, in studies using exenatide[36] or ANP,[32] although cGMP-PKG signalling has been shown to be blocked in the presence of hypercholesterolaemia in rats.[105] It is becoming clear that in addition to cardiomyocytes, cardioprotection should also target other cardiac or circulating cell types including endothelium, pericytes, smooth muscle, nerves, platelets, neutrophils, mast cells, fibroblasts, and resident stem cells[106–108](see *Figure 3*). These may provide direct or paracrine benefits, for example via production of exosomes. Similarly, other physiological aspects of acute IRI are emerging as potential targets, including oedema[109] and microvascular dysfunction and obstruction.[108]

A crucial issue is timing. Ischaemic time is a critical determinant of cardiomyocyte death and the latter is exacerbated by reperfusion injury. Most evidence suggests that cardioprotective pathways must be targeted during the first minutes of reperfusion.[110–112] Similar to the wave-front of injury occurring during ischaemia, there is believed to be a wave-front of injury during reperfusion. Indeed, several early studies in dogs and rabbits suggested that MI size increases during the early hours of reperfusion up until 48 h, suggesting that reperfusion injury may remain a therapeutic target during this time.[113–115] Although several successful examples of this approach have been published,[116–119] the concept remains

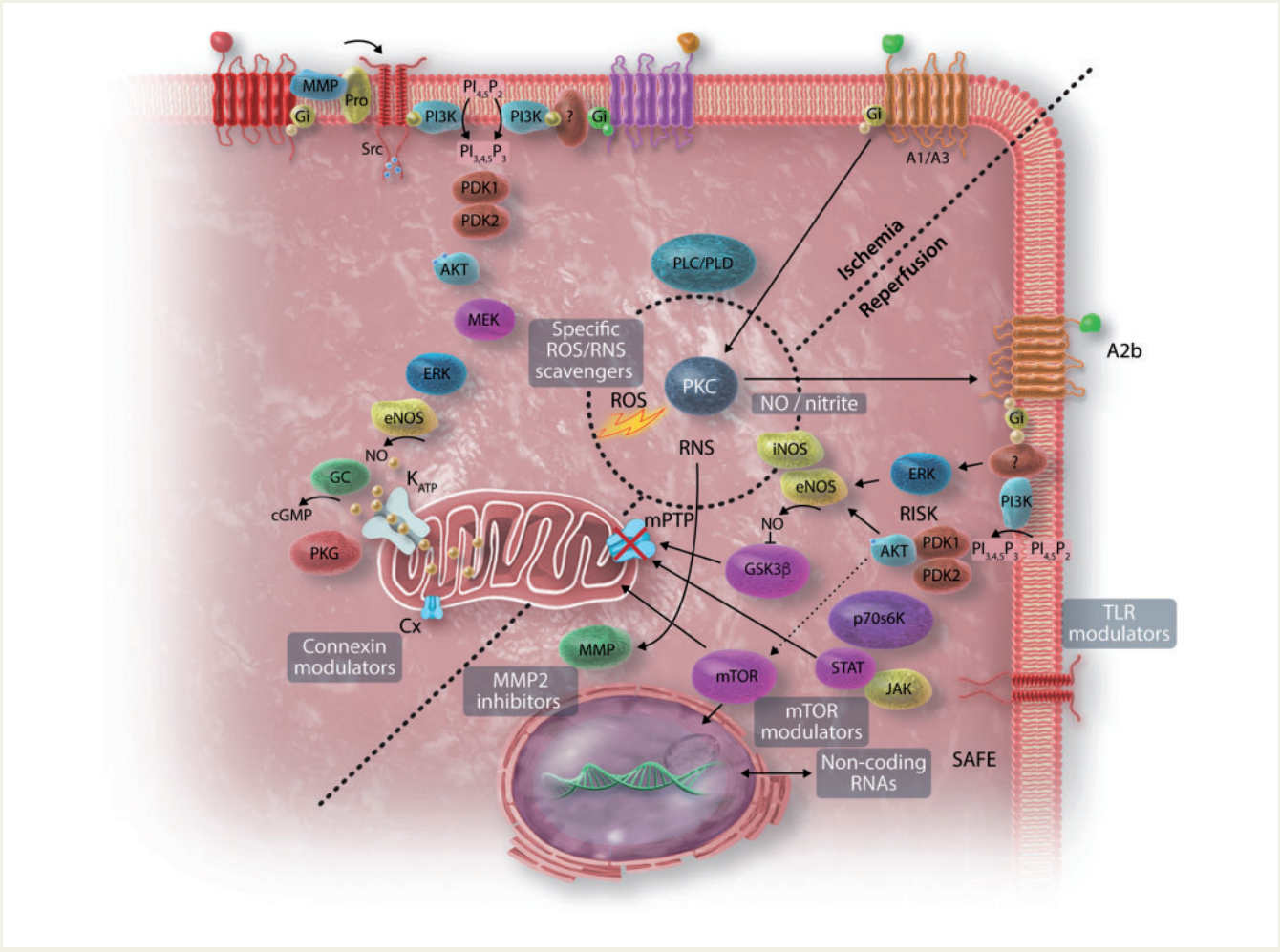

Figure 3 Promising new targets for cardioprotection: ROS scavengers, NO/nitrite, non-coding RNAs, Cx stimulators, MMP inhibitors, TLR modulators, mTOR signalling modulators? (the background image on NO-cGMP-PKG, RISK and SAFE pathways has been modified from[213]).

Table 2 Checklist of criteria to consider when identifying a functionally important therapeutic target for clinical translation

Is the target present and functional at or before reperfusion?
Has the target been validated in large animal models that simulate the clinical setting?
Has the target been validated in human myocardium?
Is the target affected by age or gender?
Is the target functional in the presence of co-morbidities and co-medications (including anaesthetics)?
Is the target amenable to drug-based or physical manipulation?
Is the appropriate drug concentration achieved within limits of toxicity?
Is the target appropriate in isolation or should it be combined with another target (i.e. broad spectrum approach)?

somewhat controversial. Whether or not late reperfusion injury can be targeted is an important but unresolved question, as are the targets of such late reperfusion injury.

In identifying a new target for cardioprotection, crucial, but frequently overlooked steps are to prove the presence of the target in the heart and its activation (or downregulation) at or before early reperfusion (Table 2). When considering a therapeutic target, its presence in humans must be kept in focus. For example, cardiac expression of some receptors can differ between rodents and humans, as for GLP-1R.[120,121] In addition, rodents may differ from humans regarding the relative importance of intracellular pathways such as RISK and SAFE pathways.[122] Validation of a target in the myocardium of the target patient population can be challenging, but *ex vivo* organ-bath models such as the human atrial-appendage model can be informative in this regard.[123,124] A therapeutic target must remain valid in the setting of current clinical practise, specifically in the complex settings of PCI and

cardiac surgery, the latter of which already incorporates cardioprotective strategies such as cardioplegia and hypothermia.

In addition to targets mentioned above, novel therapeutic targets currently under investigation include the immune system (particularly monocytes, macrophages, extracellular DNA and RNA, inflammasomes), platelet—inflammatory cell interactions, exosomes and microvesicles, G-protein coupled receptor (GPCRs), Toll-like receptors (TLRs), and proteases such as MMPs and calpains.[107,125] It may be time to look beyond the mitochondrial PTP to other mitochondrial targets such as the mitochondrial calcium uniporter, mitochondrial fission and fusion proteins, Connexin 43/20, mitochondrial metabolism and mitophagy, and to understand the crosstalk between the mitochondria and the sarcoplasmic reticulum (SR). The pathways of caloric restriction including sirtuins and mammalian target of rapamycin (mTOR) present interesting potential targets. Thinking towards the future, other therapeutic pathways that would be likely to be of enormous benefit include the prophylactic stimulation of new collateral vessels, drugs that can simulate the benefits of exercise, or—perhaps even more optimistically—treatments that stimulate cardiac regeneration or reverse the age-related phenotype,[126] as was recently, and controversially, suggested for GDF11.[127,128]

A checklist of important criteria when considering target development is included in *Table 2*. An overriding consideration is whether a single target is likely to be effective in isolation, or whether multi-targeted approaches are more consistent with the multiple mechanisms of IRI,[51,129] a question which will be discussed in the following section.

3.1 Multi-omics strategies to identify novel therapeutic targets and signalling pathways in an unbiased way

Since the pathophysiology of IHD and cardioprotection is extremely complex, it is conceivable that large scale, unbiased, global approaches capable of detecting multiple branches of the signalling networks activated in the ischaemic heart with the presence of several co-morbidities and co-medications might be more successful in the search for novel therapeutic targets. High-throughput techniques now allow high-resolution, genome-wide investigation of genetic variants, epigenetic modifications, and associated gene expression profiles, as well as proteomics and metabolomics (although the latter techniques need further technological development). These techniques offer simultaneous readouts of hundreds of proteins and metabolites in an unbiased, non-hypothesis driven way. 'Omics' analyses usually provide a huge amount of information requiring large data storage, advanced computational resources and complex bioinformatics tools. The possibility of integrating different 'omics' approaches into 'multi-omics' gives new hope to better understand the signalling network responsible for IHD and cardioprotection.[130,131]

As an example, metabolomic profiling of biological samples from patients during myocardial IRI[132–134] has highlighted specific metabolic 'profiles' that might be used to identify novel biomarkers or therapeutic targets.[135–138] Using a comparative metabolomic approach, Chouchani et al.[83] discovered an evolutionarily conserved biochemical 'fingerprint' of ischaemia characterized by elevated intracellular levels of succinate, an intermediate of the citric acid cycle. Selective accumulation of succinate is a universal metabolic signature of ischaemia in several tissues and cell types, enhancing mitochondrial ROS production during reperfusion[83,84] and promoting tissue inflammation.[139] Preventing succinate accumulation and/or oxidation might represent a novel and more effective target for cardioprotection.[84,85]

4. New treatment strategies for cardioprotection

4.1 Combination therapy—multi-targeted approach directed to different intracellular signalling pathways within the cardiomyocyte

Many of the cardioprotective strategies which have failed in the clinical setting have relied upon using a single-targeted approach, directed to one specific molecule or intracellular signalling pathway. However, a multi-targeted approach directed to more than one intracellular signalling pathways may be a more effective cardioprotective strategy, especially if one of the signalling cascades is impaired due to the presence of a co-morbidity such as diabetes.[140] A number of experimental studies have investigated the cardioprotective effect of combining one or more ischaemic conditioning strategies. Some studies have demonstrated a synergistic effect between RIC and IPost,[141,142] a finding which has been replicated in the clinical setting with a reduction in MI size with RIC and IPost combined but no cardioprotective effect with IPost alone.[59] This may suggest that although some of the signalling cascades are shared between RIC and IPost, there are sufficient differences to mediate a synergistic cardioprotective effect.

It may also be possible to combine the use of 'old' drugs to repurpose them for cardioprotection, such that the combination may have new or greater efficacy than the component drugs alone. The combination of adenosine and lidocaine may be an example. Each component alone has equivocal or controversial efficacy, but has greater efficacy with some new actions when combined in caridoplegic solution.[143]. However, MI size reduction by combined adenosine and lidocaine has always remained controversial.[144,145] Most recently, it has been shown that combining limb RIC with insulin or insulin mimetics (such as exenatide) has a synergistic effect in terms of reducing MI size in the porcine model of acute MI, and this was demonstrated to be mediated by targeting 2 different pro-survival intracellular signalling pathways.[146] This therapeutic approach will be tested in the COMBAT-MI trial (NCT02404376) which will investigate whether combining RIC with exenatide is more effective that either treatment alone in terms of reducing MI size in reperfused STEMI patients.

4.2 Combination therapy—multi-targeted approach directed to other players in IRI

Since cell death caused by acute myocardial IRI occurs as a result of the combined action of multiple cellular players in cardiac tissue (i.e. cardiomyocytes, microvasculature, fibroblasts, inflammatory cells, and platelets), additive protection might be achieved from a multi-targeted approach directed to different cell types. This may be achieved using either one agent known to have two different unrelated targets or two or more agents in combination directed to two or more different unrelated targets.

4.2.1 Coronary microvasculature- endothelial cells, vascular smooth muscle cells, and pericytes

Microvascular injury due to microembolic obstruction of the coronary microcirculation may amplify the damage caused by the obstruction of the epicardial arteries and nullify the result of reperfusion therapies in STEMI patients.[147–150] The contractile phenotype of vascular smooth muscle cell (VSMC) secretes adiponectin, a compound also shown to be

cardioprotective.[151] However, VSCMCs as well as fibroblasts might transform under stress to the synthetic phenotype and to myofibroblasts, respectively.[152] Preliminary experimental data have implicated a potential role of pericytes as mediators of microvascular obstruction following AMI.[153] In addition, the pericardium has been also suspected to be involved in acute myocardial IRI.[154]

4.2.2 Platelets

Anti-thrombotic therapy is a cornerstone in post-reperfusion therapy. Platelet activation is a consequence of endothelial injury, and activation of platelet adhesion and aggregation increase cell death independently of any effect on myocardial flow and microvascular obstruction.[155,156] Thromboxane A2, e.g. has been reported to activate cardiac afferent nerves and promote a sympathetic cardiac response.[157] Moreover, platelets are the source of multiple bioactive components including extracellular vesicles released into the bloodstream with the potential to affect cells and tissue at a distance.[158] Recent experimental data have demonstrated that the platelet P2Y12 inhibitors are able to reduce MI size when administered at the onset of reperfusion via 'conditioning' signalling pathways.[159–161] Although IPost provided no added protection over that achieved with a P2Y12 inhibitor alone, hypothermia or a sodium-hydrogen exchanger did induce additional protection.[161]

4.2.3 Fibroblasts

Cardiac fibroblasts are an essential component of cardiac tissue and constitute about 11% of total cell numbers in the adult heart.[162] Cardiac fibroblasts can originate from primary mesenchymal cells, from circulating cells such as mesenchymal stem cells or through endothelial-mesenchymal transition.[163] Thus, cardiac fibroblasts represent a heterogeneous cell population with distinct developmental origin, which may also determine their basal functions as well as their responses to stress such as IRI. Cardiac fibroblasts produce the extracellular matrix and secrete cytokines, chemokines and growth factors, and thereby interact with cardiomyocytes. For example: hypoxic fibroblast-conditioned medium enhanced the susceptibility of cardiomyocytes to ROS-induced mitochondrial permeability transition opening and reduced cardiomyocyte viability.[164] The adenosine triphosphate (ATP) release by cardiomyocytes through the large conductance channel pannexin 1 is involved in the early phase of fibroblast activation during ischaemia.[165] The low molecular weight isoform of fibroblast growth factor (FGF) 2 is released from the adult mouse heart during IR and mediates cardioprotective effects during IRI independent from its pro-angiogenic effects even when delivered only during reperfusion.[166,167] In response to myocardial IRI in the mouse, FGF21, another member of the FGF family of growth factors, is upregulated and released from adipocytes (and from hepatocytes) into the circulation and induces cardioprotective effects.[168] Fibroblasts and their involvement in post-infarct inflammation can serve a cardioprotective function.[169] Thus, there is a close interplay between cardiomyocytes and fibroblasts in IRI and protection from it.

4.2.4 Inflammation

Acute IRI in the setting of an AMI induces an initial inflammatory response (the purpose of which is to remove necrotic debris from the MI zone), followed by an anti-inflammatory phase which permits wound healing to occur. The transition between these two phases is orchestrated by a finely regulated but complex interaction between multiple players within the heart itself (including cardiomyocytes, endothelial cells, fibroblasts) and components of the immune response (including neutrophils, platelets, monocytes, macrophages, dendritic cells and lymphocytes).[170–172] Treatment addressing inflammation has been disappointing overall, and as such, newer treatments or the use of combination therapy are needed to target novel inflammatory mediators of acute IRI such as inflammasomes,[173] extracellular nucleic acids (RNA, DNA),[174,175] and neutrophil extracellular traps,[176] in order to attenuate the initial inflammatory response and/or upregulate the anti-inflammatory response to acute IRI.

4.2.5 Nerves

Local sensory innervation of the heart was shown in the 1990s to play a crucial role in IPC,[177] myocardial function, and the transcriptomic profile of the heart.[178] Autonomic reflexes and the autonomic nerve terminals introduce variability in response to IRI in the human heart. The sympathetic nerve terminals also participate in paracrine signalling in the heart as well. Norepinephrine, neuropeptide-y, calcitonin gene-related peptide and ATP have all been proposed to have a direct cardioprotective potential.[179] Presynaptic beta-receptors might facilitate release of these mediators.[180] The widespread use of beta blockade in the clinical setting and the proposed role of the vagal nerve[181] in RIC[182] reflect our lack of complete understanding of the details of innervation in the human heart and the impact of innervation on acute IRI.

4.2.6 Extracellular vesicles

Unfortunately, so far the knowledge on the interaction between the different cell types within the cardiac tissue as well as on inter-organ communication is very limited. Extracellular vesicles (exosomes and microvesicles) are potential players in intercellular and inter-organ communication.[183] Accordingly, exosomes have been shown as potential players of cardioprotection by RIC.[158] However, it needs to be established if therapy by extracellular vesicles may confer cardioprotection.[184]

5. Optimizing the design of experimental studies to improve the translation of cardioprotection into the clinical setting

Most proof-of-concept and confirmatory experimental studies were performed in healthy and young animals, and demonstrated a reduction of irreversible myocardial injury by ischaemic conditioning interventions.[185] In addition, the AMI model most often relies upon external occlusion of a healthy coronary artery, whereas in patients, AMI is an inflammatory condition heralded by the rupture of an atherosclerotic plaque. However the extent of protection varied depending on the animal species, the experimental set-up (including the algorithm of the conditioning stimulus,[186] the extent and duration of the sustained (index) ischaemia, the mode of reperfusion, anaesthesia, etc.).[103] Subsequently, many investigators realized that many of the signalling pathways involved in the protection by ischaemic conditioning interventions[19,130,185,187] are also affected by sex, age, the presence of pre-existing coronary artery disease, co-morbidities and co-medications (again depending on the severity and duration of the disease and/or co-medication).[52,187] Furthermore, some co-medications *per se* can reduce the extent of irreversible myocardial injury, thereby making the delineation of any additional cardioprotective effect by ischaemic conditioning strategies difficult.[188] *Table 3* provides a summary of the co-morbidities (such as

Table 3 Summary of major counfounders reported to influence the cardioprotective effiacy of ischaemic conditioning

Confounders	Animal studies on conditioning	Human trials on conditioning
Age	Young	Middle aged, old
Co-morbidities		
0	Most	Rare
1	Some	Some
>1	None	Most
Duration of disease and co-morbidities	Short	Long
Co-medications for co-morbidities		
0	Most	Rare
1	Some	Some
>1	None	Most
Acute treatments related to intervention	None	Most (except CABG)
Anaesthesia	Most	Some (CABG)
Endpoints		
Function	Many	Many
Infarct size	Most	Many
Prognosis	Rare	Rare, mostly retrospective

hypertension, LV hypertrophy, hypercholesterolemia, diabetes, etc.) and co-medications used to treat co-morbidties which can confound cardioprotection and illustrates how these have been taken into account in experimental and clinical studies of cardioprotection. Although most animal experiments on IRI and protection from it were performed in young and otherwise healthy (therefore un-treated) animals, patients recruited into clinical cardioprotection trials are usually of advanced age and have numerous co-morbidities and related co-medications as well as acute treatments related to AMI. Therfore, more studies in adequate animal models, more closely mimicking the clinical situation, are required.

Indeed, aging[189] and many co-morbidities (mostly of short duration, such as LV hypertrophy, hyperlipidaemia or diabetes) attenuated or completely abrogated the cardioprotective effect of interventions when compared with healthy animals[187]; however, it should be noted that most of the (single, individual) co-morbidities were again induced in young animals, thereby not mimicking what does normally occur in humans (except for type 1 diabetes or homozygous familiar hypercholesterolemia). Furthermore, in animal experiments co-morbidities usually remained untreated, again not reflecting what is normally observed in clinical practise where patients will receive at least some medication (although many of them are not treated according to guidelines and to target values).

When comparing animal studies to patients undergoing CABG surgery, anaesthesia *per se* might be a confounding factor for the results obtained by cardioprotective interventions. In fact, propofol in contrast to isoflurane specifically abrogated the protection by RIC interventions.[190–193] Also, patients undergoing CABG surgery in contrast to animals will receive cardioplegia, which impacts on the extent of irreversible injury *per se* and might affect signal transduction pathways. On the other hand, patients suffering an AMI undergoing PCI will not receive anaesthetics but instead will receive anti-platelet therapy (some of which acts directly as a cardioprotectant[194–196]), which is not normally applied in animal experiments.

Another major shortcoming of animal studies is the lack of long-term follow-up of the benefits of conditioning interventions. Most animal studies determine MI size, extent of arrhythmias or contractile dysfunction between 2 and 24 h after the onset of reperfusion and the beneficial effect of conditioning on left LV remodelling and subsequent mortality is largely unknown, although of utmost clinical relevance.[8,19]

There is significant inter-species variability[53] in signalling events leading to cardioprotection by ischaemic conditioning in healthy or diseased animals, and it remains to be established whether signalling events demonstrated to be involved in most animal species can easily be transferred to cardioprotection obtained by conditioning interventions in humans.

Where do we stand?—Conditioning interventions protect young and healthy hearts from subsequent IRI of almost all animal species. Age and more or less acutely induced (single) co-morbidities or administered co-medications attenuate the observed beneficial effect of conditioning interventions. Of note, however, in patient studies, *post-hoc* analyses reveal that apart from age, none of the co-morbidities and co-medications found to be of importance in animal experiments significantly attenuate the cardioprotection obtained by conditioning interventions[197–199]; whether these discrepant findings are related to the fact that medical treatment of co-morbidities normally occuring in patients blunts their otherwise detrimental effect or whether the involved signalling pathways differ between animals and humans remains unanswered at present. Finally, the neutral result of clinical trials may be explained in many cases by the insufficient, inconsistent pre-clinical data on the investigated interventions.

6. Optimizing the design of clinical studies to improve the translation of cardioprotection

In this section, we review the major factors which may have contributed to the neutral results of recent clinical cardioprotection studies in STEMI patients (*Table 1*[215–229]) and propose strategies for optimizing the design

of future clinical studies, in order to improve the translation of cardioprotection.

6.1 Only investigate those therapies which have shown robust and consistent cardioprotection in experimental studies

In many cases, the clinical study may have been neutral because it tested a therapy which had shown inconsistent cardioprotection in experimental studies. Furthermore, the experimental data may have been limited to small animal models of acute myocardial IRI (such as mice, rats and rabbits), and lacked testing in clinically relevant large animal MI models of acute myocardial IRI (such as pig and dog).[200]

As such, future clinical studies should only test those therapies which have clearly demonstrated robust and consistent cardioprotection in both small and large animal models of acute myocardial IRI including at least one or more major comorbidities and co-medications (see later).[187]

6.2 Adoption of a multi-targeted approach to cardioprotection

In many cases, the clinical study may have been neutral because it was based on a pharmacological strategy directed to a single target, an approach which may be ineffective given that acute myocardial IRI is a complex process with different signalling cascades and multiple cellular players (cardiomyocytes, endothelial cells, fibroblasts, inflammatory cells, platelets).

As such, a multi-targeted approach using a combination of therapies may be a more effective approach to cardioprotection in the clinical setting.

6.3 Inclusion of STEMI patients most likely to benefit from a cardioprotective therapy

In many cases, the clinical study may have been neutral because it included an unselected cohort of patients. This may have included STEMI patients less likely to benefit from a novel cardioprotective therapy administered prior to PPCI, such as those with pre-PPCI TIMI flow ≥2 (patients who have spontaneously reperfused prior to PPCI),[112] and a small AAR (right and circumflex coronary artery STEMI)[201] or longer ischaemic times (up to 12 h).[202]

As such, future clinical studies should select those STEMI patients presenting with: a completely occluded coronary artery (pre-PPCI TIMI flow ≤1), a large AAR [≥30% of the LV, usually proximal or mid left anterior descending (LAD) STEMI], and shorter ischaemic times (≤4 h). However, this will clearly impact on study feasibility in terms of reducing the number of eligible patients for inclusion in the study.

6.4 Optimize the timing of the cardioprotective therapy

In some cases, the clinical study may have been neutral because of the incorrect timing of the intervention. For example, although experimental data had suggested that therapeutic hypothermia was only effective when applied prior to the index ischaemia and not at the onset of reperfusion,[203] clinical studies tested therapeutic hypothermia as a cardioprotective strategy at the time of reperfusion. In order to prevent myocardial reperfusion injury, which occurs in the first few minutes of reperfusion, it is essential to apply the cardioprotective intervention prior to PPCI; most clinical studies have taken heed of this but it is unclear whether or not the dose achieved is optimal at the time of reperfusion.

As such, future clinical studies should take into account the results of experimental studies with respect to timing of the cardioprotective therapy.

6.5 Optimize the dose of the cardioprotective therapy

In many cases, the clinical study may have been neutral because of an incorrect dose of the cardioprotective therapy. It is clear from experimental studies that the dose of the novel therapy can impact on its cardioprotective efficacy.[186,204] In most cases the most effective dose of the novel cardioprotective therapy has not been optimized in either experimental or clinical studies—crucially there is an obvious lack of phase II studies in the field of cardioprotection.

The optimum dose for cardioprotection in experimental studies must be determined and adequate phase 2 dosing clinical studies be undertaken in order to increase the likelihood of translating cardioprotection into the clinical setting.

6.6 Take into account the confounding effects of co-morbidities and co-medications given to STEMI patients

In many cases, the clinical study may have been neutral because of multiple comorbidities, and co-medications that are commonly given to STEMI patients treated by PPCI the presence of which may have either attenuated the beneficial effects of the cardioprotective therapy or might have induced cardioprotection themselves. These include drugs such as nitrates, P2Y12 platelet inhibitors, statins, opioids, and so on, all of which have been shown to exert cardioprotection by themselves and thereby mask any additional beneficial effects of endogenous cardioprotective strategies such as ischaemic conditioning.[159–161,187] However, in future clinical cardioprotection studies, it will not be possible to omit co-medications such as platelet inhibitors, given that they are essential for the management of STEMI patients treated by PPCI. What can be done is to test the proposed cardioprotective therapy in animals treated with these co-medications to ensure an additive effect can be achieved.[161]

As such, experimental studies should take into account comorbidities and co-medications when testing novel cardioprotective therapies (also see section 6).

6.7 Use relevant endpoints for cardioprotection

In some cases, the clinical study may have been neutral because of the wrong choice of endpoint used to assess the cardioprotective efficacy of the novel therapy. In proof-of-concept clinical studies of cardioprotection in STEMI patients, acute MI size measured by serum cardiac biomarkers, myocardial SPECT or more recently CMR, has been used to assess the cardioprotective efficacy of novel therapies. For assessing long-term effects of cardioprotection, echocardiography and CMR have been used to assess final MI size and adverse LV remodelling (LV volumes and ejection fraction). Although myocardial salvage (AAR subtract MI size) is a more sensitive measure than absolute reduction in MI size for assessing cardioprotection, there is currently no generally accepted and available *in vivo* measure of the AAR in reperfused STEMI patients. Myocardial SPECT is the only validated measure of myocardial salvage, and it has been utilized in multiple randomized clinical trials. However, SPECT is logistically challenging, expensive, and includes radiation

exposure. Limitations include: No distinction between new and old perfusion defects; lack of resolution to detect subendocardial infarcts; and requirement for two examinations. T2-weighted CMR has been more recently proposed to retrospectively delineate the AAR in reperfused STEMI patients although there is controversy over the use of oedema-based AAR by T2-weighted CMR.[205] As such, the most robust measurement for acute MI size is mass of new late gadolinium contrast enhancement on CMR as a percentage of LV mass. After establishing efficacy with a particular intervention, it is necessary to demonstrate improved clinical outcomes before changing clinical practise. In clinical outcome studies of cardioprotection in STEMI patients, it is essential to focus on endpoints such as cardiac death and hospitalization for heart failure which are more relevant to cardioprotection, although one may consider also potential vascular effects of ischaemic conditioning on other MACCE such as repeat MI and coronary revascularization. Furthermore, how concomitant microvascular disease (hypertension, diabetes, rheumatoid arthritis) affects the techniques that are used for endpoint evaluation in humans is not known and requires further investigation.

Although in this section we list those factors which should be taken into consideration when designing clinical cardioprotection studies, this may not always be possible or feasible in the clinical setting, highlighting the challenges in trying to balance optimizing study design and clinical reality.

7. Recommendations for improving future experimental cardioprotection studies

As discussed in the earlier chapters, most patients suffering from acute myocardial IRI are of advanced age and have multiple co-morbidities, including hypertension, LV hypertrophy, hypercholesterolemia, diabetes, have had a previous MI with subsequent LV remodelling, have developed heart failure, or all of the above. Given their multiple co-morbidities, patients also receive extensive chronic medication [β-blockers, angiotensin converting enzyme inhibitors, AT1 (angiotensin II type 1)-receptor antagonists, L-type calcium channel antagonists, statins, sulfonylureas, metformin, GLP-1-antagonists, aspirin, etc]. In addition, during the acute ischaemic event they will probably receive nitrates, P2Y12-receptor antagonists, and opioids.[18,187]

These patients may or may not benefit from cardioprotective interventions, but the prediction of protection derived from experimental research is difficult since adequate animal models mimicking the clinical scenario do not exist and are difficult to develop.[19] As such, the translation from bench to bedside could be improved if experimental studies were more appropriately designed[206]; e.g. by the selection of an adequate animal species: there is no doubt that a large animal model of MI that better mimics the clinical situation (taking into account sex, age, co-morbidities, co-medications and long term reperfusion models).[200] Furthermore, selection bias and publication of only positive results should be avoided which could be achieved by pre-registration of experimental studies (like done in clinical trials). Also in experimental trials the use of appropriate statistical tests needs to be assured.[207] Below is a list of recommendations for studies to be performed in the experimental work-up of a novel cardioprotective therapy after target validation using *in vitro*/*ex vivo* models but prior to testing in the clinical setting.

7.1 Recommendations

(1) *In vivo* small animal (acute and chronic MI size, heart failure development, mortality).
(2) *In vivo* large animal model of acute myocardial IRI (acute and chronic MI size, heart failure development, mortality).
(3) Investigate whether age or treated major co-morbidities such as diabetes mellitus, hypercholesterolemia, or obesity confound cardioprotection.
(4) Consider human heart tissue models of acute IRI (such as e.g. human atrial tissue, cell-based human heart tissue models or include human stem cell-derived cardiomyocytes).[126,208]
(5) Multicentre experimental testing of novel cardioprotective therapy using standardized protocols in small and large animal MI models with one or more co-morbidities (such as age and/or diabetes) (see below).

7.2 Adopting a multicentre approach to cardioprotection

Due to the competitive nature of innovation at early pre-clinical stages, collaborative pre-clinical development is challenging. Nevertheless, using a multi-centre blinded placebo-controlled approach, the NIH Consortium for Preclinical Assessment of Cardioprotective Therapies consortium[16] failed to find a reduction in MI size by sildenafil or sodium nitrite when administered at reperfusion in either mice, rabbit, or porcine MI models,[209,210] despite several single centre studies in small animal MI models reporting cardioprotection with these agents, suggesting inadequate blinding in the latter studies and that the therapies did not confer robust cardioprotection. This may explain, in part, why the corresponding clinical studies in STEMI patients failed to find a positive cardioprotective effect with sodium nitrite.[211,212] So, why have we not moved forward with such an investigative team model yet? The need for extensive funding and facilities to develop such models could only be made feasible if researchers in the field join forces together and apply for a specific large funding scheme such as HORIZON 2020. The neuroprotection field has come to the same conclusion, with the Multicentre Preclinical Animal Research Team, which is an international collaborative approach to overcome the translational roadblock in neuroprotection and neuroregeneration research, and whose overall objective was to discuss how to develop the capacity to undertake international multicentre animal studies. Thus, although pre-clinical studies may demonstrate the therapeutic potential of an intervention, clinical trials should not be initiated before their cardioprotective effects are confirmed in multi-centre pre-clinical studies.

8. Recommendations for improving future clinical cardioprotection studies

The design of the clinical cardioprotection study is crucial to the success of the study. In this section, we provide a list of recommendations for improving the translation of cardioprotection in the clinical setting for patient benefit.

8.1 Proof-of-concept efficacy Phase 2 studies in STEMI patients

- Only investigate those treatment strategies, which show robust and consistent cardioprotection in the experimental settings detailed above.
- Consider the influence of major co-morbidities and co-medications on the cardioprotective efficacy in patient selection. Pre-specified,

adequately powered, subgroup analyses may determine the effects of these confounding factors on cardioprotection. Be sure to measure haemodynamic parameters at the time of treatment as well as time of reperfusion.
- Where possible use multicentre randomized placebo-controlled double blind trial design.
- Only include STEMI patients with the following inclusion criteria:
  - <4 h of ischaemic symptom onset.
  - Large AAR (e.g. proximal to mid-LAD STEMI).
  - Completely occluded coronary artery (pre-PPCI TIMI flow ≤1) with post-PPCI TIMI flow > 2.
  - Consider excluding patients with significant coronary collateralization to the AAR as this may attenuate the cardioprotective effects of the therapy.
  - Consider including high-risk STEMI patients with cardiogenic shock, if technically possible, given that they benefit most from a cardioprotective therapy.
- Consider phase 2 studies to optimize the most effective dose before testing for clinical efficacy.
- Ensure that the therapy is administered prior to reperfusion and that it achieves therapeutic concentrations at the time of PPCI.
- Use clinical endpoints which are relevant to cardioprotection for acute studies (i.e. acute and chronic MI size, adverse LV remodelling (LV size and ejection fraction).

8.2 Clinical outcome Phase 3 studies in STEMI patients

As above plus

- Use clinical endpoints which are relevant to cardioprotection for clinical outcome studies i.e. cardiac death and hospitalization for heart failure.

9. Conclusions

The translation of cardioprotection into the clinical setting for patient benefit has been both challenging and disappointing. However, the failure to find a cardioprotective therapy despite 30 years of research should not put into doubt the existence of myocardial IRI as a viable target for cardioprotection, but should rather highlight the difficulties in translating novel cardioprotective therapies from the over-simplified animal MI models we all use into the complex clinical reality of a reperfused STEMI patient. Therefore, in order to improve the translation of cardioprotection into the clinical setting, we need to improve the design of the experimental and clinical studies, and in this Position Paper we have proposed some recommendations for working towards this. However, the feasibility of achieving this has to be counterbalanced by the reality of undertaking experimental and clinical MI studies.

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