

This is the peer reviewed version of the following article:

García Del Blanco B, Otaegui I, Rodríguez-Palomares JF, Bayés-Genis A, Fernández-Nofrerías E, Vilalta Del Olmo V, Carrillo X, Ibáñez B, Worner F, Casanova J, Pueo E, González-Juanatey JR, López-Pais J, Bardají A, Bonet G, Fuertes M, Rodríguez-Sinovas A, Ruiz-Meana M, Inserte J, Barba I, Gómez-Talavera S, Martí G, Serra B, Bellera N, Ojeda-Ramos M, Cuellar H, Valente F, Carmona M, Miró-Casas E, Marsal JR, Sambola A, Lidón RM, Bañeras J, Elízaga J, Padilla F, Barrabés JA, Hausenloy DJ, Ferreira-González I, García-Dorado D. (2021). Effect of COMBinAktion therapy with remote ischemic conditioning and exenatide on the Myocardial Infarct size: a two-by-two factorial randomized trial (COMBAT-MI). *Basic Res Cardiol*, 116(1), 4. doi: 10.1007/s00395-021-00842-2

which has been published in final form at: <https://doi.org/10.1007/s00395-021-00842-2>

Effect of COMBINAtion Therapy with remote ischemic conditioning and exenatide on the Myocardial Infarct size: a two-by-two factorial randomized trial (COMBAT-MI)

Bruno García del Blanco^{a,b}, MD, PhD, Imanol Otaegui^{a,b}, MD, José F. Rodríguez-Palomares^{a,b}, MD, PhD, Antoni Bayés-Genis^{b,c}, MD, PdD, Eduard Fernández-Nofrerías^{b,c}, MD, PdD, Victoria Vilalta del Olmo^{b,c}, MD, Xavier Carrillo^{b,c}, MD, PdD, Borja Ibáñez^{b,d,e}, MD, PhD, Fernando Worner^f, MD, PhD, Juan Casanova^f, MD, PhD, Eva Pueo^f, MD, Jose R. González-Juanatey^g, MD, PhD, Javier López-Pais^g, MD, Alfredo Bardají^h, MD, PhD, Gil Bonet^h, MD, Mónica Fuertes^h, MD, Antonio Rodríguez-Sinovás^{a,b}, PhD*, Marisol Ruiz-Meana^{a,b}, PhD, Javier Inserte^{a,b}, PhD, Ignasi Barba^{a,b}, PhD, Sandra Gómez-Talavera^{b,d,e}, MD, PhD, Gerard Martí^{a,b}, MD, Bernat Serra^{a,b}, MD, Neus Bellera^{a,b}, MD, PhD, Manuel Ojeda-Ramos^{a,b}, MD, Hug Cuellarⁱ, MD, PhD, Filipa Valente^{a,b}, MD, Maria Ángeles Carmona^{a,b}, Elisabet Miró Casas^{a,b} BsC, Josep R. Marsal^{a,j}, PhD, Antonia Sambola^{a,b}, MD, PhD, Rosa M. Lidón^{a,b}, MD, Jordi Bañeras^{a,b}, MD, PhD, Jaime Elízaga^{b,k}, MD, PhD, Ferran Padilla^l, MD, PhD, José A. Barrabés^{a,b}, MD, PhD, Derek J. Hausenloy^{m,n,o,p,q}, MD, PhD, Ignacio Ferreira-González^{a,j}, MD, PhD*, David García-Dorado^{a,b,†}, MD, PhD.

† In the memory of Professor Garcia-Dorado, who passed away on August 16, 2019.

- a. Cardiology Department, Vall d'Hebron Institut de Recerca (VHIR), Vall d'Hebron Hospital Universitari, Vall d'Hebron Barcelona Hospital Campus. Barcelona, Spain.
- b. CIBERCV, Instituto de Salud Carlos III, Madrid, Spain
- c. Institut de Cor, Hospital Univeristari Germans Trias i Pujol, Badalona, Spain.
- d. Centro Nacional de Investigaciones Cardiovasculares Carlos III (CNIC)
- e. IIS-Fundación Jiménez Díaz, Madrid, Spain
- f. Hospital Universitari Arnau de Vilanova. Lleida. IRBLLEIDA, Spain
- g. Hospital Clínico Universitario de Santiago de Compostela, Spain
- h. Servicio de Cardiología, Hospital Joan XXIII, Tarragona; Spain
- i. Institut Diagnostic per la Imatge, Hospital Vall d'Hebron, Barcelona, Spain.
- j. Ciber de Epidemiologia y Salud Pública, CIBERESP. Instituto de Salud Carlos III, Madrid, Spain.
- k. Hospital General Universitario Gregorio Marañón, Madrid, Spain
- l. Cardiology Department. Hospital Universitari Mútua Terrassa. Terrassa. Spain.
- m. Cardiovascular & Metabolic Disorders Program, Duke-National University of Singapore Medical School, Singapore.
- n. National Heart Research Institute Singapore, National Heart Centre, Singapore.
- o. Yong Loo Lin School of Medicine, National University Singapore, Singapore.
- p. The Hatter Cardiovascular Institute, University College London, London, UK.
- q. The National Institute of Health Research University College London Hospitals Biomedical Research Centre, Research & Development, London, UK.

*Corresponding authors. Address for correspondence:

Antonio Rodríguez-Sinovás. antonio.rodriquez.sinovas@vhir.org

Ignacio Ferreira-González. iferreir@vhebron.net

Cardiology Department, Vall d'Hebron Hospital. Passeig Vall d'Hebron 119-129. 08035. Barcelona. Spain. Phone: 932746134; Fax: 923746063

Acknowledgements: The COMBAT-MI trial was conceived, designed and led by Prof. David Garcia-Dorado and represents the last contribution of his scientific and medical career dedicated entirely to the search for new and effective cardioprotective strategies that can benefit patients with ischemic heart disease. With this article we want to pay a heartfelt tribute to his memory and express our gratitude to him. The authors also want to thank Prof. Aurora García-Dorado for her valuable and expert assistance in the statistical analysis. The trial was sponsored with a grant from Instituto de Salud Carlos III (PIE 13/00027) and a grant from Generalitat de Catalunya (PERIS SLT/2381/2016).

Author contributions:

Besides Prof. David Garcia-Dorado, all authors contributed to the study conception and design. Material preparation and data collection was performed by: García del Blanco, Otaegui, Rodríguez-Palomares, Bayés-Genis, Fernández-Nofrerías, Vilalta del Olmo, Carrillo, Ibáñez, Worner, Casanova, Pueo, González-Juanatey, López-Pais, Bardají, Gil Bonet Fuertes, Gómez-Talavera, Martí, Carmona, Miró, Serra, Bellera, Ojeda-Ramos, Cuellar, Valente, Sambola, Lidón, Bañeras, Elízaga Padilla, Barrabés.

Analyses were performed by Marsal, Ferreira-González and García-Dorado.

The first draft of the manuscript was written by Ignacio Ferreira-González and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

ABSTRACT.

Remote Ischemic Conditioning (RIC) and the GLP-1 analog exenatide activate different cardioprotective pathways and may have additive effects on infarct size (IS). Here, we aimed to assess the efficacy of RIC as compared with sham procedure, and of exenatide, as compared with placebo, and the interaction between both, to reduce IS in humans. We designed a two-by-two factorial, randomized controlled, blinded, multicenter, clinical trial. Patients with ST-segment-elevation myocardial infarction receiving primary percutaneous coronary intervention (PPCI) within 6 hours of symptoms were randomized to RIC or sham procedure and exenatide or matching placebo. The primary outcome was IS measured by late gadolinium enhancement in cardiac magnetic resonance performed 3-7 days after PPCI. The secondary outcomes were myocardial salvage index, transmural index, left ventricular ejection fraction and relative microvascular obstruction volume. A total of 378 patients were randomly allocated and, after applying exclusion criteria, 222 patients were available for analysis. There were no significant interactions between the two randomization factors on the primary or secondary outcomes. IS was similar between groups for the RIC ($24\pm 11.8\%$ in the RIC group vs $23.7\pm 10.9\%$ in the sham group, $p=0.827$) and the exenatide hypotheses ($25.1\pm 11.5\%$ in the exenatide group vs $22.5\pm 10.9\%$ in the placebo group, $p=0.092$). There were no effects with either RIC or exenatide on the secondary outcomes. Unexpected adverse events or side effects of RIC and exenatide were not observed. In conclusion, neither RIC nor exenatide, or its combination, were able to reduce IS in STEMI patients when administered as an adjunct to PPCI.

Keywords: Remote ischemic conditioning, exenatide, ST-segment-elevation acute myocardial infarction, primary percutaneous coronary intervention.

INTRODUCTION

Prompt reperfusion with primary percutaneous coronary intervention (PPCI) in patients with ST-segment elevation myocardial infarction (STEMI) is the most effective therapy for limiting myocardial infarct size (IS). However, there are some shortcomings in the daily clinical practice. On the one hand, the time window for effective IS reduction is narrow [14]. On the other hand, reperfusion itself can induce further myocardial injury accounting up to 50% of the final IS [49], a phenomenon known as ischemia/reperfusion injury (IRI) [33].

Among the many approaches that have been proposed to limit IRI in patients with STEMI, remote ischemic conditioning (RIC) and the administration of glucagon-like peptide-1 (GLP-1) analogs appeared especially attractive [15]. RIC has been considered until very recently a promising strategy, since in many clinical studies conducted in STEMI patients it increased myocardial salvage and reduced IS by 20–30% when applied before or during reperfusion [5,7,10,34,35,45,48]. However, the cardioprotective effect of RIC is currently under debate. In a recently published randomized clinical trial, RIC had no effect on clinical outcomes or on IS evaluated by cardiac biomarkers [18]. This discrepancy has been attributed to the use of different RIC protocols or to a potential type I error in those trials that showed cardioprotective effects [18].

GLP-1 is an incretin hormone that decreases blood glucose by stimulating insulin production and secretion in response to nutrient intake. The GLP-1 receptor promotes glucose uptake by myocardial cells improving their metabolic efficiency [44,50], and activates different cardioprotective biochemical pathways against IRI [3,31,37]. GLP-1 analogs, such as exenatide and liraglutide, are currently used to reduce blood glucose in type 2 diabetic patients, and it has been reported their usefulness for reducing IS in preclinical animal models of acute IRI [41,43] and in proof-of-concept clinical trials [6,29,46].

Emerging data suggest that reduction of IRI may require the additive or synergistic effects of multitarget strategies [8]. A study conducted by our group showed that exenatide and RIC activate different cardioprotective pathways and had additive effects on IS reduction after transient coronary occlusion in pigs [24]. However, it is unknown

whether this combined effect of RIC and exenatide also occurs in patients when administered during the acute phase of STEMI. For this reason, we conducted the COMBAT-MI trial (a two-by-two factorial, randomized controlled, multicenter, clinical trial) to assess the efficacy of RIC as compared with a sham procedure, and the GLP-1 analog exenatide, as compared with placebo, and the combined effect of both strategies to reduce IS assessed by cardiac magnetic resonance (CMR) in patients with STEMI receiving PPCI.

METHODS

The COMBinAtion Therapy in Myocardial Infarction (COMBAT-MI, registered at www.clinicaltrials.gov (NCT02404376); EudraCT number, [2015-001000-58](https://eudract.eu/number/2015-001000-58).) was an investigator-initiated, prospective, randomized, multicenter, two-by-two factorial, double blinded, clinical trial, comparing RIC with a sham procedure and exenatide with matching placebo in patients with STEMI undergoing PPCI. The study was conducted in accordance with the principles of the 1964 Declaration of Helsinki and its later amendments and the European guidelines for Good Clinical Practice, and was approved by the *Agencia Española de Medicamentos y Productos Sanitarios* (AEMPS) and the Ethics Committees of the participant institutions. Data from 6 tertiary academic centers in Spain were included. All participants provided written informed consent before randomization.

Patient selection and randomization.

Patients eligible for enrolment were ≥ 18 years old with a diagnosis of STEMI characterized by chest pain or other ischemic symptoms and ≥ 1 mm ST elevation in ≥ 2 leads in the same territory or ≥ 2 mm ST elevation in ≥ 2 V1 through V4 leads or left bundle branch block with ≥ 1 mm concordant ST elevation, and presenting within 6 hours of symptom onset.

Exclusion criteria were known hypersensitivity to exenatide or any of excipients, contraindication to CMR, assumed life expectancy below one year, pregnancy, ongoing participation in another trial, clinical instability that led to inability to study comprehension (i.e. loss of consciousness, confusion, profound cardiogenic shock) and TIMI flow grade > 1 at the time of diagnostic coronary angiography.

Allocation to treatments in each participating center was done via a web-based clinical trial support system accessible 24h a day (W3NEXUS, Barcelona Spain). Stratification was internally determined by each participating center using permuted blocks with sizes of 4, 6 or 8. Patients eligible were enrolled in the emergency room or upon entering the catheterization laboratory and were randomly assigned to one of four groups according to 1:1:1:1 ratio. Table 1 shows patient distribution in all four groups.

Study procedures.

In the RIC procedure, an automated cuff (autoRIC™, CellAegis Devices, partner CELL; Toronto; Canada) was placed on the upper arm and inflated to 200mmHg for 5 min and then deflated for 5 min, a cycle which was programmed to be undertaken 4 times in total, as previously described [5]. The RIC protocol was applied trying to start at least 20 min before artery aperture (balloon inflation or stent deployment) and all patients completed the 4 cycles. For the control group, a sham cuff was placed on the upper arm that simulated the autoRIC™ device with the same sound and vibration. The total number of inflations at which coronary blood flow was restored was registered.

In the exenatide arm, an intravenous infusion of exenatide (18µg) diluted in saline (vehicle, 180mL) was intravenously administered, prior to the PPCI, at a flow rate of 72mL/h (0.12µg/min). After 15 min, the flow rate was reduced to 26mL/h (0.043µg/min) and maintained for additional 6 hours. This protocol was previously found to be safe and effective against IRI in STEMI patients [28]. In the matching-placebo arm, vehicle was administered following the same protocol used for exenatide infusion. Exenatide or vehicle infusions were started following randomization and before obtaining arterial access for cardiac catheterization, with no delay to application of reperfusion therapy. Only a research nurse was unblinded and prepared the treatment assigned according to the randomization process in the cath lab. The investigator team collecting and analyzing the data was blinded to treatment allocation.

Cardiac catheterization was performed by experienced operators in PPCI without any delay due to the acceptance to participate in the trial, and stenting was performed according to the usual procedures. The PPCI procedure followed guideline recommendations [25], according to local practice. Thrombectomy and selection of antiplatelet and anticoagulant regimens were per operator discretion.

CMR Protocol.

All CMR studies were performed in a Siemens or Philips 1.5 T or 3 T clinical scanner using a phased-array cardiac receiver coil at 3-7 days following the PPCI procedure and a standardized CMR image protocol. Electrocardiogram-gated breath-hold short-axis cine views were performed to quantify volumes and ejection fraction (SSFP sequences; slice thickness: 6 mm; space between slices 67%; matrix: 256x256; field of view: 300-370mm; temporal resolution <50ms). Additional 2-chamber, 3-chamber and 4-chamber views were also obtained. LGE images were acquired at identical slice positions to the cine images after the administration of 0.2 mmol/kg of body weight Gadolinium-DTPA (Gd-DTPA) (Berlex, Montville, NJ, USA).

STIR sequences were used in the same view as the cine sequences, all in mid-diastole to evaluate the edema, (slice thickness: 8mm; space between slices 20%; matrix: 256x256; FOV: 300-370mm; temporal resolution <50ms; repetition time: 2 R-R intervals; echo time: 100 ms; inversion time: 170 ms; flip angle: 160°; bandwidth, 781 Hz/pixel).

A segmented inversion-recovery (seg-IR) gradient-echo sequence was acquired starting at 25 min after contrast administration to minimize IS overestimation associated to myocardial edema as previously described [36] (Matrix 256 x 197, voxel size 2.0 x 1.6 x 6 mm, TE 4.91 ms, TR 700 ms, flip angle 30°; and the bandwidth 140 z/pixel).

The images were centrally analyzed by experts blinded to group allocation. Image analysis was conducted on a workstation (Cvi42, Circle Cardiovascular Imaging Inc., Calgary, Alberta, Canada) by 2 cardiologists specialized in cardiac imaging. Data on reproducibility, inter- and intraobserver variability have been previously published [32].

Study endpoints.

The primary study endpoint was myocardial IS measured by late gadolinium enhancement (LGE) in a CMR performed 3-7 days after PPCI and expressed as a percent of the left ventricular (LV) mass.

Secondary study endpoints included: 1) myocardial salvage index (MSI), defined as ratio of LGE to the extent of myocardial edema, assessed by T2-weighted CMR; 2) the

transmurality index, defined as the ratio of the mass of myocardium showing LGE to the mass of the myocardial segment containing it; 3) the left ventricular end-diastolic volume (LVEDV) and left ventricular ejection fraction (LVEF), as determined by CMR imaging; and 4) the relative microvascular obstruction volume (MVO) compared to IS measured on LGE sequences of IS.

Sample size calculation and statistical analysis.

The trial used a factorial design to evaluate two hypotheses. One hypothesis was that RIC would be superior to a sham procedure with regard to the outcome IS. The second hypothesis was that exenatide would be superior to matching placebo with regard to the same outcome. If both hypotheses were true, a third hypothesis of an interaction effect between RIC and exenatide would be tested.

The population for the primary and secondary outcome analyses included all patients who underwent randomization, received at least one cycle of RIC intervention (or sham procedure) at the time of myocardial reperfusion, had a baseline TIMI ≤ 1 , underwent CMR within the specified timeframe and the analysis of the primary outcome in the CMR was available.

To calculate sample size, we assume a conservatively IS of 24% with a standard deviation of 14 based on previous studies. Sample size was computed to detect a reduction of IS of at least 20% with either RIC or exenatide or with the combined therapy, with a statistical power $1-\beta=0.8$ and a significance level $\alpha=0.05$. This resulted in a calculated total sample size of 274 patients. Assuming that diagnostic coronary angiography could not confirm the initial diagnosis of STEMI in 2% of patients receiving the study treatment, and that in an additional 20% TIMI flow would be >1 , a total of 351 should be included to ensure the calculated sample size. This figure was increased to 378 to allow a loss of patients with confirmed STEMI and TIMI ≤ 1 not completing an adequate CMR study.

Continuous variables were described using mean and standard deviation for normally distributed variables and median/interquartile ranges for non-normal variables. Categorical variables were described using absolute and relative frequencies. Baseline differences between groups were compared using t-Student test or exact Fisher test

where appropriate. The effect of exenatide and RIC were assessed using a linear model. Interaction between the two randomization factors (RIC and exenatide) on the primary and secondary outcomes was assessed. If there was no significant interaction, the two factors were analyzed independently. In a first step the linear model incorporated the main effect for each treatment and the interaction term for exenatide and RIC. In case of no statistical effect of the interaction, the model was readjusted including the main effects of exenatide and RIC.

Pre-specified subgroup analyses were defined based on age (< 65 years vs \geq 65 years), the infarct-related coronary artery (left anterior descending vs other), and time from symptom onset to reperfusion (< 120 min vs \geq 120 min). P values were calculated for interaction tests of prespecified subgroups. The statistical analysis was performed using RStudio.

RESULTS.

From Mar-2016 through Jun-2019 a total of 378 patients were randomized from six sites to receive RIC or a sham procedure and to receive exenatide or matching placebo. The exclusion of patients for several reasons during PPCI and hospitalization led to a final sample of 222 patients randomized to the different arms available for the analysis (Figure 1).

The characteristics of the patients at baseline were well balanced between the trial groups (Table 2). The median age among all the patients was 60.5 years, and 16.2% were women. The prevalence of cardiovascular risk factors (hypertension, dyslipidemia, diabetes mellitus, peripheral arterial disease, chronic renal impairment, chronic obstructive pulmonary disease, as well as a clinical history of MI and stroke) was similar in the study groups. There were more patients randomized to placebo who were active smokers compared to the group randomized to exenatide ($P= 0.023$). There were not relevant differences in the baseline drugs treatment.

Table 3 shows procedure details. Most patients were in a stable hemodynamic condition and on Killip-I (n=186; 86.1%), without differences among groups. There were also no differences in the delay from the initial symptoms to arrival at the catherization lab (median 137 min) or in the mean hemoglobin and creatinine values on admission. The

most common infarct-related coronary artery was the right coronary artery (n=101; 45.7%), followed by the left anterior descending coronary artery (n=80; 36.2%) and, in most instances, the initial coronary TIMI flow was 0 (n=195, 88.2%). Coronary TIMI flow grade 3 was reached in 91.4% of cases. Most patients had received 2 (n=92; 41.8%) or 3 (n=73; 33.2%) RIC cycles at the time of coronary flow restoration, without differences among groups. There were no differences between groups in the use of aspiration thrombectomy or in the medications administered during the procedure.

CMR was performed 3-7 days after PPCI (median 5 days). Table 4 shows the results for the main hypotheses on the primary and secondary outcomes. There were no significant interactions between the two randomization factors on the primary or secondary outcomes, which together with the lack of significant effect for each treatment, implies no significant effect of the combined therapy. IS was similar between groups for the RIC hypothesis (24±11.8% in the RIC group vs 23.7±10.9% in the sham procedure group, $P=0.827$), and for the exenatide hypothesis (25.1±11.5% in the exenatide group vs 22.5±10.9% in the placebo group, $P=0.092$). Furthermore, there were no differences among groups in both hypotheses in LVEDV, either considering absolute or indexed values. Accordingly, LVEF was virtually identical between groups for the RIC hypothesis (45.2±10.1% in the RIC group vs 45.9±10.4% in the sham procedure, $P=0.60$) and for the exenatide hypothesis (45.4±10.2% in the exenatide group vs 45.7±10.4% in the placebo group, $P=0.858$). There were no differences between groups for the RIC hypothesis in MSI but, in the case of the exenatide hypothesis, those patients randomized to exenatide had a lower MSI than those randomized to placebo (22.3±15.8% vs 27.9±18.1%, $P=0.018$), indicating an opposite direction of the hypothesized effect. Finally, there were no differences among groups, in both hypotheses, in the relative MVO and transmural index.

In the pre-specified subgroup analyses based on age, the infarct-related coronary artery, and time from symptom onset to reperfusion, we did not detect any effect of RIC or exenatide on IS (Figure 2).

Exploratory clinical adverse events during hospitalization were similar in all groups in the intention-to-treat analysis (Table 5) and also in the safety analysis performed on the entire randomized sample (Table 6).

DISCUSSION

This two-by-two factorial, double blinded, randomized controlled, multicenter clinical trial was designed on the hypothesis of finding a benefit from RIC and exenatide, a GLP-1 analogue, to limit the size of the myocardial infarct. On this basis, we hypothesized a possible additional benefit of the combination of both therapies when administered adjunct to PPCI. After randomizing 378 patients to the four potential strategies, no effect of RIC or exenatide was found to reduce IS in patients with STEMI submitted to PPCI.

Our factorial design approach has been based on the positive results of some previous trials that tested each separate therapy [5,7,25,35,41] and on the positive results obtained in pigs by our group in 2015 [1]. Our results suggested a protective effect of RIC and exenatide and, most importantly, supported the superiority of combination therapy over the individual treatments to reduce IS. This additive effect was based on the fact that RIC and exenatide exerted protection by different mechanisms [1]. In the case of RIC, experimental studies have demonstrated that the cardioprotective signal is transferred from the remote conditioned organ or tissue, either through the release of humoral factors or through activation of neuronal pathways, to the heart, where it triggers cardioprotective signaling cascades [19]. In our preclinical study, RIC was associated with a reduction of nitro-oxidative stress in a pig model of transient coronary occlusion. Protection by GLP-1 analogs has been associated with modulation of glycolytic metabolism [23], a fact that was confirmed in our previous study using ¹H-NMR [1].

The results of our experimental study could not be replicated in the clinical setting. Many reasons, extensively reviewed elsewhere [4,8,20,21,26], have been proposed to explain the failure to translate experimental results to patients' cardioprotection. Also, some conceptual and technical errors in the design of clinical trials have contributed to this translation failure [4,16,20,21]. For instance, most preclinical research on both RIC and GLP-1 analogs has been carried out in young and healthy animals, in the absence of medications, commonly used in STEMI patients and that can modify the efficacy of both cardioprotective strategies [4,17,26,27]. Particularly important are the P2Y₁₂ antagonists, which are nowadays widely used as routine anti-platelet therapy and induce

protection *per se* [2,47]. In the present study we have followed the guidelines for rigor and reproducibility of clinical studies as published by Botker et al. [4]. According to these authors, the gold standard for clinical trial design is a prospective, randomized, blinded, controlled study, with infarct size measured by CMR as the ideal primary endpoint [4], characteristics that have been fulfilled in this work.

In the specific case of RIC, we applied the same protocol that was used in a proof-of-concept trial, with the difference that in such study RIC was started in the ambulance [5] whereas we initiated RIC at hospital arrival. Thus, the number of complete RIC cycles before coronary flow restoration in that trial was four, whereas in our study only 17.6% of patients completed the entire protocol at the time of reperfusion, which may explain in part the lack of benefit of RIC as compared with the proof-of-concept trial. However, comparisons with that trial should be made cautiously, since the assessment of the MSI was done at 30 days of reperfusion and it was estimated by gated single photon emission tomography. In addition, prior research has shown that physiologic biochemical responses appear right after the first inflation [9].

Regarding exenatide, our protocol was based on that described by Lønberg et al [29], aimed to attain plasma concentrations of the GLP-1 analogue in a range previously shown to be beneficial (0.03 to 0.30 nmol/L) [38]. We have not quantified plasma concentration of exenatide in the present study, although it seems reasonable to think that it should be within the same range that in Lønberg's study, as the protocol was the same. Nevertheless, the possibility that the therapeutic level was not optimal cannot be excluded.

A major concern that could be related with the different results between studies is the lack of uniformity in the method used to quantify IS. In some studies it has been based on repolarization changes during STEMI (ST-segment deviation scores) or on the pattern of serum cardiac biomarkers release as surrogate markers [18,34,35,48]. These were unblinded studies, which could have led to some bias. Even considering only those studies that used imaging techniques to assess different strategies to decrease IRI, heterogeneity is also present. Four studies have shown benefit of RIC to reduce IS by imaging. However, they were relatively small size studies, their RIC protocols differed and, most importantly, they considered different endpoints [5,7,10,45]. In line with a

recent consensus document [24], we have used CMR-based IS (extent of LGE) as the primary outcome measure. In our study, the ratio of LGE to edema was included as a surrogate for myocardial salvage index. Data arising after the design of the trial demonstrated that edema is not an accurate surrogate for area at risk [11], especially when cardioprotective therapies are applied [12]. Indeed, CMR-based myocardial salvage index is no longer recommended in trials assessing the effect of cardioprotective therapies [42]. In any case, as we found no differences in the LGE to edema ratio, the selection of this secondary outcome had no impact on the trial results.

Different mechanisms depending on the method used to assess IS with CMR could justify the divergences among these studies. To start with, methods for IS quantification have differed, and have included manual planimetry [7], Otsu method [24,45], full width at half maximum [40], or standard deviations [10] as it was in our case. Another key point is the time elapsed from the contrast administration to the LGE sequence acquisition. Some studies have shown that this time interval is crucial in the acute phase due to the presence of myocardial edema, and that IS estimation can vary up to 20% depending on this time-interval [30,36]. In our study protocol, LGE sequences were acquired 25 minutes after contrast administration to minimize this effect. Other studies with a similar methodology [10,24] did not show any differences in IS post-RIC when the acquisition was delayed up to 15-20 minutes post-contrast. In the same manner, the absence of edema reduction in our study, as compared with other studies [7,40,45], could also reflect differences in the time of performing CMR after PPCI, since edematous reaction is bimodal [13].

After the results of Hausenloy's study [18], which was an appropriately powered trial, a potential impact of RIC alone on short or medium term clinically relevant outcomes seems unlikely. However, it has been reported that the combination of intrahospital RIC and ischemic postconditioning increases myocardial salvage assessed by CMR compared to conventional PPCI [10] and reduces the rate of major adverse cardiac events and heart failure after long-term follow-up [39]. These results suggest that in addition to the excellent results achieved with contemporary treatment of STEMI, the implementation of optimized conditioning protocols may further improve clinical outcome of STEMI patients. It has been proposed that future trials on cardioprotection should focus on patients who really need adjunctive therapies, including those with

Killip class ≥ 3 , where there is still room for improved clinical outcomes, as well as on patients with limited access to modern reperfusion therapies (i.e., developing countries) [21,22].

The present study has several limitations. First of all, the number of patients with >1 TIMI flow at the initial coronary angiogram was unexpectedly high. Therefore, the total number of patients available for the primary analysis was 222, whereas the calculated total sample size was 274 patients. A post-hoc analysis based on the results obtained with 222 patients revealed as unlikely a type II error (1.1% for the exenatide hypothesis, 7.1% for the RIC hypothesis and 0.1% for the interaction hypothesis). As early mentioned, a significant proportion of patients (41.8%) had only completed 2 cycles of the autoRIC protocol at the time of coronary flow restoration, which could limit the efficacy of the protection. We did a post-hoc subgroup analysis considering only those patients who had received at least 3 cycles at the moment of coronary flow restoration (n=117), and no benefit was observed either (data not shown). Finally, we decided not to include the pattern of cardiac biomarkers release as an endpoint because it is only a surrogated outcome of the actual IS and would require uniform measurement in a central laboratory to achieve good accuracy, which was not possible in this study. In any case, in a subanalysis of 146 patients with valid data from the coordinator center, there were also no differences between the groups in the peak median values of CK-MB neither in the RIC hypothesis (median 261.85, Interquartile range [141.85 - 337.15] in the RIC group and 234.2 [174 - 353.6] in the sham group; p=0.43) nor in the exenatide hypothesis (266.1 [174 - 345.4] in the exenatide group and 229.95 [145.6 - 330.9] in the placebo group; p=0.33).

In conclusion, the results of our trial suggest no benefit on IS reduction in the short term when RIC, exenatide or both therapies combined are administered as an adjunct to PPCI. Whether a medium or long-term benefit on myocardial function and remodeling could be observed in some patients should be explored in future clinical trials.

DECLARATIONS:

Funding The trial was sponsored with a grant from Instituto de Salud Carlos III (PIE 13/00027) and a grant from Generalitat de Catalunya (PERIS SLT/2381/2016). The sponsors have not been involved in the design, conduct, collection, analysis, interpretation of the data, nor in the preparation, review, or approval of the manuscript.

Conflicts of interest Authors declare that they have no conflict of interest.

Ethics approval This study was conducted in accordance with the principles of the 1964 Declaration of Helsinki and its later amendments and the European guidelines for Good Clinical Practice and was approved by the *Agencia Española de Medicamentos y Productos Sanitarios* (AEMPS) and the Ethics Committees of the participant institutions.

Consent to participate All participants provided written informed consent before randomization.

Consent for publication The manuscript is not submitted elsewhere nor is under consideration for publication. All authors have read and agreed with the submission of the manuscript.

Availability of data and material All data and materials used for this study are displayed or can be displayed upon request.

FIGURE LEGENDS:

Figure 1. Study flow chart. (A): Remote ischemic conditioning (RIC) vs sham remote ischemic conditioning. (B) Exenatide vs matching placebo. Abbreviations: CMR: Cardiac magnetic resonance; PPCI: Primary percutaneous coronary intervention; STE: ST-segment elevation.

Figure 2. Forest plot representing the estimated effect on the relative necrotic mass (%) for pre-specified subgroup analyses of the primary endpoint in the intention-to-treat population. Abbreviations: LAD= Left anterior descending.

References

1. Albuquerque-Bejar JJ, Barba I, Inserte J, Miro-Casas E, Ruiz-Meana M, Poncelas M, Vilarrosa U, Valls-Lacalle L, Rodriguez-Sinovas A, Garcia-Dorado D (2015) Combination therapy with remote ischaemic conditioning and insulin or exenatide enhances infarct size limitation in pigs. *Cardiovasc Res* 107:246-254 doi:10.1093/cvr/cvv171
2. Barrabes JA, Inserte J, Mirabet M, Quiroga A, Hernando V, Figueras J, Garcia-Dorado D (2010) Antagonism of P2Y12 or GPIIb/IIIa receptors reduces platelet-mediated myocardial injury after ischaemia and reperfusion in isolated rat hearts. *Thromb Haemost* 104:128-135 doi:10.1160/TH09-07-0440
3. Bose AK, Mocanu MM, Carr RD, Brand CL, Yellon DM (2005) Glucagon-like peptide 1 can directly protect the heart against ischemia/reperfusion injury. *Diabetes* 54:146-151 doi:10.2337/diabetes.54.1.146
4. Botker HE, Hausenloy D, Andreadou I, Antonucci S, Boengler K, Davidson SM, Deshwal S, Devaux Y, Di Lisa F, Di Sante M, Efentakis P, Femminò S, García-Dorado D, Giricz Z, Ibanez B, Iliodromitis E, Kaludercic N, Kleinbongard P, Neuhauser M, Ovize M, Pagliaro P, Rahbek-Schmidt M, Ruiz-Meana M, Schlüter KD, Schulz R, Skyschally A, Wilder C, Yellon DM, Ferdinandy P, Heusch G (2018) Practical guidelines for rigor and reproducibility in preclinical and clinical studies on cardioprotection. *Basic Res Cardiol* 113:39- doi: 10.1007/s00395-018-0696-8
5. Botker HE, Kharbanda R, Schmidt MR, Bottcher M, Kaltoft AK, Terkelsen CJ, Munk K, Andersen NH, Hansen TM, Trautner S, Lassen JF, Christiansen EH, Krusell LR, Kristensen SD, Thuesen L, Nielsen SS, Rehling M, Sorensen HT, Redington AN, Nielsen TT (2010) 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 doi:10.1016/S0140-6736(09)62001-8
6. Chen WR, Hu SY, Chen YD, Zhang Y, Qian G, Wang J, Yang JJ, Wang ZF, Tian F, Ning QX (2015) Effects of liraglutide on left ventricular function in patients with ST-segment elevation myocardial infarction undergoing primary percutaneous coronary intervention. *Am Heart J* 170:845-854 doi:10.1016/j.ahj.2015.07.014
7. Crimi G, Pica S, Raineri C, Bramucci E, De Ferrari GM, Klersy C, Ferlini M, Marinoni B, Repetto A, Romeo M, Rosti V, Massa M, Raisaro A, Leonardi S, Rubartelli P, Oltrona VL, Ferrario M (2013) 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 doi:10.1016/j.jcin.2013.05.011
8. Davidson SM, Ferdinandy P, Andreadou I, Botker HE, Heusch G, Ibañez B, Ovize M, Schulz R, Yellon DM, Hausenloy DJ, Garcia-Dorado D (2019) Multitarget Strategies to Reduce Myocardial Ischemia/Reperfusion Injury: JACC Review Topic of the Week. *J Am Coll Cardiol* 73:89-99 doi:10.1016/j.jacc.2018.09.086
9. Dezfulian C, Taft M, Corey C, Hill G, Krehel N, Rittenberger JC, Guyette FX, Shiva S (2017) Biochemical signaling by remote ischemic conditioning of the arm

versus thigh: Is one raise of the cuff enough? *Redox Biol* 12:491-498
doi:10.1016/j.redox.2017.03.010

10. Eitel I, Stiermaier T, Rommel KP, Fuernau G, Sandri M, Mangner N, Linke A, Erbs S, Lurz P, Boudriot E, Mende M, Desch S, Schuler G, Thiele H (2015) Cardioprotection by combined intrahospital remote ischaemic preconditioning and postconditioning in ST-elevation myocardial infarction: the randomized LIPSIA CONDITIONING trial. *Eur Heart J* 36:3049-3057 doi:10.1093/eurheartj/ehv463
11. Fernandez-Jimenez R, Barreiro-Perez M, Martin-Garcia A, Sanchez-Gonzalez J, Agüero J, Galan-Arriola C, Garcia-Prieto, Diaz-Pelaez, Vara, Martinez, Zamarro, Garde, Sanz, Fuster, Sanchez P, Ibanez (2017) Dynamic Edematous Response of the Human Heart to Myocardial Infarction: Implications for Assessing Myocardial Area at Risk and Salvage. *Circulation* 136:1288-1300
doi:10.1161/CIRCULATIONAHA.116.025582
12. Fernandez-Jimenez R, Galan-Arriola C, Sanchez-Gonzalez J, Agüero J, Lopez-Martin GJ, Gomez-Talavera S, Garcia-Prieto J, Benn A, Molina-Iracheta A, Barreiro-Perez M, Martin-Garcia A, Garcia-Lunar I, Pizarro G, Sanz J, Sanchez PL, Fuster V, Ibanez B (2017) Effect of Ischemia Duration and Protective Interventions on the Temporal Dynamics of Tissue Composition After Myocardial Infarction. *Circ Res* 121:439-450 doi:10.1161/CIRCRESAHA.117.310901
13. Fernandez-Jimenez R, Garcia-Prieto J, Sanchez-Gonzalez J, Agüero J, Lopez-Martin GJ, Galan-Arriola C, Molina-Iracheta A, Doohan R, Fuster V, Ibañez B (2015) Pathophysiology Underlying the Bimodal Edema Phenomenon After Myocardial Ischemia/Reperfusion. *J Am Coll Cardiol* 66:816-828
doi:10.1016/j.jacc.2015.06.023
14. Garcia-Dorado D, Rodríguez-Sinovas A, Ruiz-Meana M, Inserte J (2014) Protection against myocardial ischemia-reperfusion injury in clinical practice. *Rev Esp Cardiol (Engl Ed)* 67:394-404 doi: 10.1016/j.rec.2014.01.010
15. Hausenloy DJ, Barrabes JA, Botker HE, Davidson SM, Di Lisa F, Downey J, Engstrom T, Ferdinandy P, Carbrera-Fuentes HA, Heusch G, Ibanez B, Iliodromitis EK, Inserte J, Jennings R, Kalia N, Kharbanda R, Lecour S, Marber M, Miura T, Ovize M, Perez-Pinzon MA, Piper HM, Przyklenk K, Schmidt MR, Redington A, Ruiz-Meana M, Vilahur G, Vinten-Johansen J, Yellon DM, Garcia-Dorado D (2016) Ischaemic conditioning and targeting reperfusion injury: a 30 year voyage of discovery. *Basic Res Cardiol* 111:70- doi:10.1007/s00395-016-0588-8
16. Hausenloy DJ, Botker HE, Condorelli G, Ferdinandy P, Garcia-Dorado D, Heusch G, Lecour S, van Laake LW, Madonna R, Ruiz-Meana M, Schulz R, Sluijter JP, Yellon DM, Ovize M (2013) 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 doi:10.1093/cvr/cvt004
17. Hausenloy DJ, Garcia-Dorado D, Botker HE, Davidson SM, Downey J, Engel FB, Jennings R, Lecour S, Leor J, Madonna R, Ovize M, Perrino C, Prunier F, Schulz R, Sluijter JPG, van Laake LW, Vinten-Johansen J, Yellon DM, Ytrehus K, Heusch G, Ferdinandy P (2017) Novel targets and future strategies for acute cardioprotection: Position Paper of the European Society of Cardiology Working Group on Cellular Biology of the Heart. *Cardiovasc Res* 113:564-585
doi:10.1093/cvr/cvx049
18. Hausenloy DJ, Kharbanda RK, Moller UK, Ramlall M, Aaroe J, Butler R, Bulluck H, Clayton T, Dana A, Dodd M, Engstrom T, Evans R, Lassen JF, Christensen EF, Garcia-Ruiz JM, Gorog DA, Hjort J, Houghton RF, Ibanez B, Knight R,

- Lippert FK, Lonborg JT, Maeng M, Milasinovic D, More R, Nicholas JM, Jensen LO, Perkins A, Radovanovic N, Rakhit RD, Ravkilde J, Ryding AD, Schmidt MR, Riddervold IS, Sorensen HT, Stankovic G, Varma M, Webb I, Terkelsen CJ, Greenwood JP, Yellon DM, Botker HE (2019) Effect of remote ischaemic conditioning on clinical outcomes in patients with acute myocardial infarction (CONDI-2/ERIC-PPCI): a single-blind randomised controlled trial. *Lancet* 394:1415-1424 doi:10.1016/S0140-6736(19)32039-2
19. Hausenloy DJ, Yellon DM (2016) Ischaemic conditioning and reperfusion injury. *Nat Rev Cardiol* 13:193-209 doi: 10.1038/nrcardio.2016.5
 20. Heusch G (2017) Critical Issues for the Translation of Cardioprotection. *Circ Res* 120:1477-1486 doi:10.1161/CIRCRESAHA.117.310820
 21. Heusch G (2020) Myocardial ischaemia-reperfusion injury and cardioprotection in perspective. *Nat Rev Cardiol* 17:773-789 doi: 10.1038/s41569-020-0403-y
 22. Heusch G, Gersh BJ (2020) Is Cardioprotection Salvageable? *Circulation* 141:415-417 doi: 10.1161/CIRCULATIONAHA.119.044176
 23. Huisamen B, Genade S, Lochner A (2008) Signalling pathways activated by glucagon-like peptide-1 (7-36) amide in the rat heart and their role in protection against ischaemia. *Cardiovasc J Afr* 19:77-83
 24. Ibanez B, Aletras AH, Arai AE, Arheden H, Bax J, Berry C, Bucciarelli-Ducci C, Croisille P, Dall'Armellina E, Dharmakumar R, Eitel I, Fernandez-Jimenez R, Friedrich MG, Garcia-Dorado D, Hausenloy DJ, Kim RJ, Kozerke S, Kramer CM, Salerno M, Sanchez-Gonzalez J, Sanz J, Fuster V (2019) Cardiac MRI Endpoints in Myocardial Infarction Experimental and Clinical Trials: JACC Scientific Expert Panel. *J Am Coll Cardiol* 74:238-256 doi:10.1016/j.jacc.2019.05.024
 25. Ibanez B, James S, Agewall S, Antunes MJ, Bucciarelli-Ducci C, Bueno H, Caforio ALP, Crea F, Goudevenos JA, Halvorsen S, Hindricks G, Kastrati A, Lenzen MJ, Prescott E, Roffi M, Valgimigli M, Varenhorst C, Vranckx P, Widimsky P (2018) 2017 ESC Guidelines for the management of acute myocardial infarction in patients presenting with ST-segment elevation: The Task Force for the management of acute myocardial infarction in patients presenting with ST-segment elevation of the European Society of Cardiology (ESC). *Eur Heart J* 39:119-177 doi:10.1093/eurheartj/ehx393
 26. Kleinbongard P, Botker HE, Ovize M, Hausenloy DJ, Heusch G (2020) Co-morbidities and co-medications as confounders of cardioprotection-Does it matter in the clinical setting? *Br J Pharmacol* 177:5252-5269 doi: 10.1111/bph.14839
 27. Lecour S, Botker HE, Condorelli G, Davidson SM, Garcia-Dorado D, Engel FB, Ferdinandy P, Heusch G, Madonna R, Ovize M, Ruiz-Meana M, Schulz R, Sluijter JP, van Laake LW, Yellon DM, Hausenloy DJ (2014) ESC working group cellular biology of the heart: position paper: improving the preclinical assessment of novel cardioprotective therapies. *Cardiovasc Res* 104:399-411 doi:10.1093/cvr/cvu225
 28. Lonborg J, Kelbaek H, Vejstrup N, Botker HE, Kim WY, Holmvang L, Jorgensen E, Helqvist S, Saunamaki K, Terkelsen CJ, Schoos MM, Kober L, Clemmensen P, Treiman M, Engstrom T (2012) Exenatide reduces final infarct size in patients with ST-segment-elevation myocardial infarction and short-duration of ischemia. *Circ Cardiovasc Interv* 5:288-295 doi:10.1161/CIRCINTERVENTIONS.112.968388
 29. Lonborg J, Vejstrup N, Kelbaek H, Botker HE, Kim WY, Mathiasen AB, Jorgensen E, Helqvist S, Saunamaki K, Clemmensen P, Holmvang L, Thuesen L, Krusell LR, Jensen JS, Kober L, Treiman M, Holst JJ, Engstrom T (2012)

- Exenatide reduces reperfusion injury in patients with ST-segment elevation myocardial infarction. *Eur Heart J* 33:1491-1499 doi:10.1093/eurheartj/ehr309
30. Matsumoto H, Matsuda T, Miyamoto K, Shimada T, Mikuri M, Hiraoka Y (2011) Peri-infarct zone on early contrast-enhanced CMR imaging in patients with acute myocardial infarction. *JACC Cardiovasc Imaging* 4:610-618 doi:10.1016/j.jcmg.2011.03.015
 31. Meier JJ (2012) GLP-1 receptor agonists for individualized treatment of type 2 diabetes mellitus. *Nat Rev Endocrinol* 8:728-742 doi:10.1038/nrendo.2012.140
 32. Moral S, Rodriguez-Palomares JF, Descalzo M, Marti G, Pineda V, Otaegui I, Garcia DB, Evangelista A, Garcia-Dorado D (2012) Quantification of myocardial area at risk: validation of coronary angiographic scores with cardiovascular magnetic resonance methods. *Rev Esp Cardiol (Engl Ed)* 65:1010-1017 doi:10.1016/j.recesp.2012.04.020
 33. Piper HM, Garcia-Dorado D, Ovize M (1998) A fresh look at reperfusion injury. *Cardiovasc Res* 38:291-300 doi: 10.1016/s0008-6363(98)00033-9
 34. Prunier F, Angoulvant D, Saint EC, Vermes E, Gilard M, Piot C, Roubille F, Elbaz M, Ovize M, Biere L, Jeanneteau J, Delepine S, Benard T, Abi-Khalil W, Furber A (2014) The RIPOST-MI study, assessing remote ischemic preconditioning alone or in combination with local ischemic preconditioning in ST-segment elevation myocardial infarction. *Basic Res Cardiol* 109:400-410 doi:10.1007/s00395-013-0400-y
 35. Rentoukas I, Giannopoulos G, Kaoukis A, Kossyvakis C, Raisakis K, Driva M, Panagopoulou V, Tsarouchas K, Vavetsi S, Pyrgakis V, Deftereos S (2010) Cardioprotective role of remote ischemic preconditioning in primary percutaneous coronary intervention: enhancement by opioid action. *JACC Cardiovasc Interv* 3:49-55 doi:10.1016/j.jcin.2009.10.015
 36. Rodriguez-Palomares JF, Ortiz-Perez JT, Lee DC, Bucciarelli-Ducci C, Tejedor P, Bonow RO, Wu E (2015) Time elapsed after contrast injection is crucial to determine infarct transmural and myocardial functional recovery after an acute myocardial infarction. *J Cardiovasc Magn Reson* 17:43-52 doi:10.1186/s12968-015-0139-8
 37. Seufert J, Gallwitz B (2014) The extra-pancreatic effects of GLP-1 receptor agonists: a focus on the cardiovascular, gastrointestinal and central nervous systems. *Diabetes Obes Metab* 16:673-688 doi:10.1111/dom.12251
 38. Sonne DP, Engstrom T, Treiman M (2008) Protective effects of GLP-1 analogues exendin-4 and GLP-1(9-36) amide against ischemia-reperfusion injury in rat heart. *Regul Pept* 146:243-249 doi:10.1016/j.regpep.2007.10.001
 39. Stiermaier T, Jensen JO, Rommel KP, Waha-Thiele S, Fuernau G, Desch S, Thiele H, Eitel I (2019) Combined Intrahospital Remote Ischemic Preconditioning and Postconditioning Improves Clinical Outcome in ST-Elevation Myocardial Infarction. *Circ Res* 124:1482-1491 doi: 10.1161/CIRCRESAHA.118.314500
 40. Thuny F, Lairez O, Roubille F, Mewton N, Rioufol G, Sportouch C, Sanchez I, Bergerot C, Thibault H, Cung TT, Finet G, Argaud L, Revel D, Derumeaux G, Bonnefoy-Cudraz E, Elbaz M, Piot C, Ovize M, Croisille P (2012) Post-conditioning reduces infarct size and edema in patients with ST-segment elevation myocardial infarction. *J Am Coll Cardiol* 59:2175-2181 doi:10.1016/j.jacc.2012.03.026
 41. Timmers L, Henriques JP, de Kleijn DP, Devries JH, Kemperman H, Steendijk P, Verlaan CW, Kerver M, Piek JJ, Doevendans PA, Pasterkamp G, Hofer IE (2009) Exenatide reduces infarct size and improves cardiac function in a porcine

- model of ischemia and reperfusion injury. *J Am Coll Cardiol* 53:501-510
doi:10.1016/j.jacc.2008.10.033
42. Verouhis D, Sorensson P, Gourine A, Henareh L, Persson J, Saleh N, Settergren M, Sundqvist M, Tornvall P, Witt N, Bohm F, Pernow J (2016) Effect of remote ischemic conditioning on infarct size in patients with anterior ST-elevation myocardial infarction. *Am Heart J* 181:66-73 doi:10.1016/j.ahj.2016.08.004
 43. Wang X, Ding Z, Yang F, Dai Y, Chen P, Theus S, Singh S, Budhiraja M, Mehta JL (2016) Modulation of myocardial injury and collagen deposition following ischaemia-reperfusion by linagliptin and liraglutide, and both together. *Clin Sci (Lond)* 130:1353-1362 doi:10.1042/CS20160061
 44. Wei Y, Mojsov S (1995) Tissue-specific expression of the human receptor for glucagon-like peptide-I: brain, heart and pancreatic forms have the same deduced amino acid sequences. *FEBS Lett* 358:219-224 doi:10.1016/0014-5793(94)01430-9
 45. White SK, Frohlich GM, Sado DM, Maestrini V, Fontana M, Treibel TA, Tehrani S, Flett AS, Meier P, Ariti C, Davies JR, Moon JC, Yellon DM, Hausenloy DJ (2015) Remote ischemic conditioning reduces myocardial infarct size and edema in patients with ST-segment elevation myocardial infarction. *JACC Cardiovasc Interv* 8:178-188 doi:10.1016/j.jcin.2014.05.015
 46. Woo JS, Kim W, Ha SJ, Kim JB, Kim SJ, Kim WS, Seon HJ, Kim KS (2013) 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 doi:10.1161/ATVBAHA.113.301586
 47. Yang XM, Liu Y, Cui L, Yang X, Liu Y, Tandon N, Kambayashi J, Downey JM, Cohen MV (2013) Platelet P2Y₁₂ blockers confer direct postconditioning-like protection in reperfused rabbit hearts. *J Cardiovasc Pharmacol Ther* 18:251-262 doi:10.1177/1074248412467692
 48. Yellon DM, Ackbarkhan AK, Balgobin V, Bulluck H, Deelchand A, Dhuny MR, Domah N, Gaoneadry D, Jagessur RK, Joonas N, Kowlessur S, Lutchoo J, Nicholas JM, Pauvaday K, Shamloll O, Walker JM, Hausenloy DJ (2015) Remote Ischemic Conditioning Reduces Myocardial Infarct Size in STEMI Patients Treated by Thrombolysis. *J Am Coll Cardiol* 65:2764-2765 doi:10.1016/j.jacc.2015.02.082
 49. Yellon DM, Hausenloy DJ (2007) Myocardial reperfusion injury. *N Engl J Med* 357:1121-1135 doi: 10.1056/NEJMra071667
 50. Zhao T, Parikh P, Bhashyam S, Bolukoglu H, Poornima I, Shen YT, Shannon RP (2006) Direct effects of glucagon-like peptide-1 on myocardial contractility and glucose uptake in normal and postischemic isolated rat hearts. *J Pharmacol Exp Ther* 317:1106-1113 doi:10.1124/jpet.106.100982

Table 1. Number of patients in the four treatment groups.

	Sham RIC	RIC
Matching placebo	58	54
Exenatide	62	48

Table 2: Baseline characteristics of the patients.

	RIC	Sham RIC	<i>P</i> Value	Exenatide	Matching Placebo	<i>P</i> Value	Total
Number of patients, no.	102	120		110	112		222
Age, yr, (mean ± SD)	62.2±10.9	60.8±11.6	0.339	61.7±11.5	61.2±11	0.771	61.5±11.3
Female, no. (%)	14 (13.7)	22 (18.3)	0.368	22 (20)	14 (12.5)	0.148	36 (16.2)
BMI, kg/m ² , (mean ± SD)	27.2±3.9	27.5±4	0.652	27.6±3.9	27.1±4	0.390	27.4±3.9
Active Smoker, no. (%)	48 (47.1)	54 (45)	0.788	42 (38.2)	60 (53.6)	0.023	102 (45.9)
Hypertension, no. (%)	46 (45.1)	57 (47.5)	0.787	45 (40.9)	58 (51.8)	0.109	103 (46.4)
Dyslipidemia, no. (%)	58 (56.9)	69 (57.5)	1.000	60 (54.5)	67 (59.8)	0.498	127 (57.2)
Medically treated diabetes, no. (%)	24 (23.5)	23 (19.2)	0.510	22 (20)	25 (22.3)	0.743	47 (21.2)
Peripheral arterial disease, no. (%)	9 (8.8)	6 (5)	0.292	10 (9.1)	5 (4.5)	0.191	15 (6.8)
Renal impairment (Cr <50), no. (%)	5 (4.9)	3 (2.5)	0.475	2 (1.8)	6 (5.4)	0.280	8 (3.6)
COPD, no. (%)	6 (5.9)	7 (5.8)	1.000	5 (4.5)	8 (7.1)	0.569	13 (5.9)
Previous AMI, no. (%)	1 (1)	3 (2.5)	0.627	0 (0)	4 (3.6)	0.122	4 (1.8)
Previous stroke, no. (%)	2 (2)	1 (0.8)	0.595	2 (1.8)	1 (0.9)	0.620	3 (1.4)
Medications at admission for PPCI, no. (%)							
Statins	27 (26.5)	26 (21.7)	0.432	25 (22.7)	28 (25)	0.754	53 (23.9)
Antiplatelet therapy	11 (10.8)	13 (10.8)	1.000	11 (10)	13 (11.6)	0.830	24 (10.8)
Beta blockers	13 (12.7)	12 (10)	0.531	12 (10.9)	13 (11.6)	1.000	25 (11.3)
ACEI or ARB	32 (31.4)	37 (30.8)	1.000	28 (25.5)	41 (36.6)	0.083	69 (31.1)
Metformin	13 (15.3)	18 (17.8)	0.595	19 (21.1)	12 (12.5)	0.620	31 (16.7)

Abbreviations: ACEI= Angiotensin converting enzyme inhibitor; AMI= Acute Myocardial Infarction; ARB= angiotensin receptor blocker; BMI= Body mass index; COPD= Chronic Obstructive Pulmonary Disease; RIC= Remote ischemic conditioning.

Table 3: Procedure details.

	RIC	Sham RIC	P Value	Exenatide	Matching Placebo	P Value	Total
Infarct-related artery, no.	102	119	0.478	110	111	0.934	221
Left anterior descending, no. (%)	35 (34.3)	45 (37.8)		38 (34.5)	42 (37.8)		80 (36.2)
Right coronary artery, no. (%)	47 (46.1)	54 (45.4)		53 (48.2)	48 (43.2)		101 (45.7)
Circumflex, no. (%)	10 (9.8)	11 (9.2)		11 (10)	10 (9)		21 (9.5)
Other, no. (%)	10 (9.8)	9 (7.6)		8 (7.3)	11 (9.9)		19 (8.6)
TIMI flow grade at admission							
TIMI=0, no. (%)	89 (87.3)	106 (89.1)		96 (88.1)	99 (88.4)		195 (88.2)
TIMI=1, no. (%)	13 (12.7)	13 (10.9)		13 (11.9)	13 (11.6)		26 (11.8)
Number of RIC cycles at reperfusion, total no.	102	118	0.931	109	111	0.960	220
1 cycles, no. (%)	5 (4.9)	6 (5.1)		6 (5.5)	5 (4.5)		11 (5)
2 cycles, no. (%)	41 (40.2)	51 (43.2)		43 (39.4)	49 (44.1)		92 (41.8)
3 cycles, no. (%)	38 (37.3)	35 (29.7)		40 (36.7)	33 (29.7)		73 (33.2)
4 cycles, no. (%)	18 (17.6)	26 (22)		20 (18.3)	24 (21.6)		44 (20)
Stenting of culprit lesion by PPCI, no. (%)	95 (96.9)	108 (96.4)	1.000	102 (98.1)	101 (95.3)	0.445	203 (96.7)
Aspiration thrombectomy, no. (%)	55 (56.7)	63 (57.8)	0.889	54 (52.9)	64 (61.5)	0.260	118 (57.3)

Table 3: Procedure details (continued)

	RIC	Sham RIC	P Value	Exenatide	Matching Placebo	P Value	Total
TIMI flow grade after procedure, total no.	102	118	0.131	109	111	0.788	220
TIMI=0, no. (%)	3 (2.9)	0 (0)		1 (0.9)	2 (1.8)		3 (1.4)
TIMI=1, no. (%)	0 (0)	1 (0.8)		1 (0.9)	0 (0)		1 (0.5)
TIMI=2, no. (%)	8 (7.8)	7 (5.9)		7 (6.4)	8 (7.2)		15 (6.8)
TIMI=3, no. (%)	91 (89.2)	110 (93.2)		100 (91.7)	101 (91)		201 (91.4)
Treatment at PPCI							
Heparin, no. (%)	77 (75.5)	97 (80.8)	0.414	85 (77.3)	89 (79.5)	0.746	174 (78.4)
Clopidogrel, no. (%)	67 (65.7)	76 (63.3)	0.779	69 (62.7)	74 (66.1)	0.674	143 (64.4)
Aspirin, no. (%)	101 (99)	117 (97.5)	0.627	108 (98.2)	110 (98.2)	1.000	218 (98.2)
Ticagrelor, no. (%)	19 (18.6)	26 (21.7)	0.618	25 (22.7)	20 (17.9)	0.406	45 (20.3)
Prasugrel, no. (%)	15 (14.7)	16 (13.3)	0.847	13 (11.8)	18 (16.1)	0.440	31 (14)
Nitrates, no. (%)	43 (42.2)	55 (45.8)	0.591	48 (43.6)	50 (44.6)	0.893	98 (44.1)

Table 3: Procedure details (continued)

	RIC	Sham RIC	<i>P</i> Value	Exenatide	Matching Placebo	<i>P</i> Value	Total
Number of patients, no.	102	120		110	112		222
CMR post PPCI, day no. (mean ± SD)	6±4.5	6.4±9.5	0.699	6.1±5.9	6.3±9	0.802	6.2±7.6
Primary Outcome							
Infarct size (%), (mean ± SD) ^a	24±11.8	23.7±10.9	0.827	25.1±11.5	22.5±10.9	0.092	23.8±11.3
Secondary Outcomes							
LVEDV (mL), (mean ± SD)	149.5±38.9	144.5±38	0.342	146.8±40.5	146.8±36.4	0.999	146.8±38.4
LVEDV/BSA, (mean ± SD)	78.7±17.9	75.4±17.9	0.167	76.2±18.5	77.6±17.4	0.550	76.9±17.9
LVESV (mL), (mean ± SD)	83.2±30.2	79.9±31.7	0.438	81.3±30.6	81.6±31.5	0.943	81.4±31
LVESV/BSA, (mean ± SD)	43.7±14.8	41.6±15.3	0.285	42.1±14.6	43.1±15.7	0.614	42.6±15.1
LVEF (%),(mean ± SD)	45.2±10.1	45.9±10.4	0.599	45.4±10.2	45.7±10.4	0.858	45.6±10.3
MSI (%),(mean ± SD) ^b	23.7±15.8	26.3±18.2	0.279	22.3±15.8	27.9±18.1	0.018	25.1±17.2
MVO (%),(mean ± SD) ^c	1±2.3	1.2±1.9	0.565	1.1±1.9	1±2.3	0.735	1.1±2.1
Transmularity Index, (mean ± SD) ^d	46.9±11.4	47.5±12.6	0.707	48.2±12.1	46.3±11.9	0.242	47.2±12
Extent of edema (gr), (mean ± SD)	37.2±16.3	37.9±16.9	0.751	37.9±17	37.3±16.3	0.780	37.6±16.6

Abbreviations: MSI=LGE/edema extent; MVO= Relative microvascular obstruction; PPCI= Primary percutaneous coronary intervention; RIC= Remote ischemic conditioning; SBP= Systolic blood pressure.

^a Percentage of LGE volume to the total of left ventricular mass.

^b Ratio of LGE to the extent of myocardial edema

^c Percentage of dark areas of absent contrast surrounded by hyper-enhanced infarct tissue to the total Infarct size.

^d Ratio of the mass of myocardium showing LGE to the mass of the myocardial segment containing it.

Table 4: Outcomes.

	RIC	Sham RIC	P Value	Exenatide	Matching Placebo	P Value	Total
Number of patients, no.	102	120		110	112		222
CMR post PPCI, day, mean \pm SD	6 \pm 4.5	6.4 \pm 9.5	0.699	6.1 \pm 5.9	6.3 \pm 9	0.802	6.2 \pm 7.6
Primary Outcome							
Infarct size (%), mean \pm SD ^a	24 \pm 11.8	23.7 \pm 10.9	0.827	25.1 \pm 11.5	22.5 \pm 10.9	0.092	23.8 \pm 11.3
Secondary Outcomes							
LVEDV (mL), mean \pm SD	149.5 \pm 38.9	144.5 \pm 38	0.342	146.8 \pm 40.5	146.8 \pm 36.4	0.999	146.8 \pm 38.4
LVEDV/BSA, mean \pm SD	78.7 \pm 17.9	75.4 \pm 17.9	0.167	76.2 \pm 18.5	77.6 \pm 17.4	0.550	76.9 \pm 17.9
LVESV (mL), mean \pm SD	83.2 \pm 30.2	79.9 \pm 31.7	0.438	81.3 \pm 30.6	81.6 \pm 31.5	0.943	81.4 \pm 31
LVESV/BSA, mean \pm SD	43.7 \pm 14.8	41.6 \pm 15.3	0.285	42.1 \pm 14.6	43.1 \pm 15.7	0.614	42.6 \pm 15.1
LVEF (%), mean \pm SD	45.2 \pm 10.1	45.9 \pm 10.4	0.599	45.4 \pm 10.2	45.7 \pm 10.4	0.858	45.6 \pm 10.3
MSI (%), mean \pm SD ^b	23.7 \pm 15.8	26.3 \pm 18.2	0.279	22.3 \pm 15.8	27.9 \pm 18.1	0.018	25.1 \pm 17.2
MVO (%), mean \pm SD ^c	1 \pm 2.3	1.2 \pm 1.9	0.565	1.1 \pm 1.9	1 \pm 2.3	0.735	1.1 \pm 2.1
Transmularity Index, mean \pm SD ^d	46.9 \pm 11.4	47.5 \pm 12.6	0.707	48.2 \pm 12.1	46.3 \pm 11.9	0.242	47.2 \pm 12
Extent of edema (gr), mean \pm SD	37.2 \pm 16.3	37.9 \pm 16.9	0.751	37.9 \pm 17	37.3 \pm 16.3	0.780	37.6 \pm 16.6

Abbreviations: BSA= Body surface area; CMR=Cardiac magnetic resonance; LVEDV= Left Ventricular End Diastolic Volume; LVESV= Left Ventricular End Systolic Volume; LGE: late gadolinium enhancement; MSI=LGE/edema extent; MVO=Relative microvascular obstruction; PPCI= Primary percutaneous coronary intervention; RIC= Remote ischemic conditioning.

^a Percentage of LGE volume to the total of left ventricular mass.

^b Ratio of LGE to the extent of myocardial edema

^c Percentage of dark areas of absent contrast surrounded by hyper-enhanced infarct tissue to the total Infarct size.

^d Ratio of the mass of myocardium showing LGE to the mass of the myocardial segment containing it.

Table 5. Exploratory adverse events during hospitalization in the sample of patients included in the intention to treat analysis.

	RIC	Sham RIC	p	Exenatide	Matching Placebo	p	Total
Number of patients; n	102	120		110	112		222
Death; n (%)	1 (1)	2 (1.7)	1.000	1 (0.9)	2 (1.8)	1.000	3 (1.4)
Myocardial Infarction; n (%)	4 (3.9)	1 (0.8)	0.183	3 (2.7)	2 (1.8)	0.682	5 (2.3)
Stroke TIA; n (%)	0 (0)	3 (2.5)	0.252	1 (0.9)	2 (1.8)	1.000	3 (1.4)
Heart Failure; n (%)	3 (2.9)	4 (3.3)	1.000	4 (3.6)	3 (2.7)	0.720	7 (3.2)
New coronary intervention; n (%)	3 (2.9)	1 (0.8)	0.336	3 (2.7)	1 (0.9)	0.367	4 (1.8)
Ventricular arrhythmia during primary PCI; n (%)	12 (11.8)	11 (9.2)	0.659	11 (10)	12 (10.7)	1.000	23 (10.4)
Atrial fibrillation or flutter during hospitalization; n (%)	3 (2.9)	9 (7.5)	0.151	5 (4.5)	7 (6.3)	0.768	12 (5.4)
Local vascular complications; n (%)	3 (2.9)	2 (1.7)	0.663	3 (2.7)	2 (1.8)	0.682	5 (2.3)
Bradycardia requiring medical treatment; n (%)	8 (7.8)	6 (5)	0.418	5 (4.5)	9 (8)	0.409	14 (6.3)
Skin allergic reaction; n (%)	1 (1)	0 (0)	0.459	0 (0)	1 (0.9)	1.000	1 (0.5)

Abbreviations: MVO=Relative microvascular obstruction; TIA = transient ischemic attack.

Table 6. Exploratory adverse events during hospitalization in entire sample of randomized patients.

	RIC	Sham RIC	p	Exenatide	Matching placebo	p	Total
Number of patients; n	187	191		189	189		378
Death; n (%)	4 (2.1)	2 (1)	0.445	3 (1.6)	3 (1.6)	1.000	6 (1.6)
Myocardial Infarction; n (%)	7 (3.7)	2 (1)	0.102	3 (1.6)	6 (3.2)	0.503	9 (2.4)
Stroke TIA; n (%)	2 (1.1)	3 (1.6)	1.000	3 (1.6)	2 (1.1)	0.686	5 (1.3)
Heart Failure; (%)	4 (2.1)	9 (4.7)	0.259	6 (3.2)	7 (3.7)	0.787	13 (3.4)
New coronary intervention; n (%)	6 (3.2)	2 (1)	0.171	2 (1.1)	6 (3.2)	0.284	8 (2.1)
Ventricular arrhythmia (VT or VF) during PPCI; n (%)	14 (7.5)	12 (6.3)	0.688	13 (6.9)	13 (6.9)	1.000	26 (6.9)
Atrial fibrillation or flutter during hospitalization; n (%)	4 (2.1)	12 (6.3)	0.071	9 (4.8)	7 (3.7)	0.622	16 (4.2)
Local vascular complications in arterial access; n (%)	5 (2.7)	2 (1)	0.280	2 (1.1)	5 (2.6)	0.449	7 (1.9)
Bradycardia requiring medical treatment; n (%)	9 (4.8)	6 (3.1)	0.441	9 (4.8)	6 (3.2)	0.600	15 (4)
Skin allergic reaction; n (%)	1 (0.5)	0 (0)	0.495	1 (0.5)	0 (0)	1.000	1 (0.3)

Abbreviations: MVO= Relative microvascular obstruction; PPCI= Primary percutaneous intervention; TIA = Transient ischemic attack.