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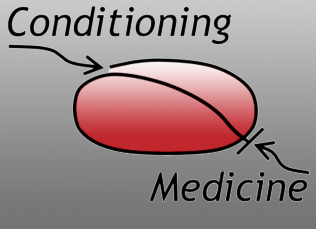

REVIEW ARTICLE | OPEN ACCESS

Realizing the therapeutic potential of novel cardioprotective therapies: The EU-CARDIOPROTECTION COST Action - CA16225

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Acute myocardial infarction (AMI) and the heart failure (HF) that often follows are the leading causes of death and disability in Europe and worldwide. As such, new treatment strategies are needed to protect the heart against acute ischemia/reperfusion injury (IRI) in order to preserve cardiac function and prevent adverse left ventricular remodeling and HF – a strategy termed "cardioprotection." Despite intensive experimental and clinical research since the discovery of the remarkable cardioprotective effect of ischemic preconditioning more than 3 decades ago, there are currently no effective cardioprotective therapies in clinical practice. The challenge has been to successfully translate novel cardioprotective therapies discovered in experimental studies into the clinical setting for patient benefit.

This EU-CARDIOPROTECTION COST Action CA16225 will address this challenge by setting up a pan-European research network of leading experts in experimental and clinical cardioprotection, to jointly develop innovative strategies for translating novel cardioprotective therapies into the clinical setting. This will be achieved through 4 main research objectives, each linked to the activities of a Working Group (WG): (1) **WG1 New Targets:** to use innovative strategies to discover novel targets for cardioprotection, given that many of the established cardioprotective targets have so far failed; (2) **WG2 Combination Therapy:** to investigate the effects of using combination therapy directed to multiple targets as an innovative cardioprotective strategy, given that single-targeted approaches to cardioprotection have so far failed; (3) **WG3 Confounders:** to use more clinically relevant animal AMI/HF models for testing novel cardioprotective therapies which take into account the confounding effects of co-morbidities and co-medications, given that many of the failed clinical studies have been based on therapies developed using juvenile healthy animal models; and (4) **WG4 Consortium:** to set up a European network of research centers (called the European Cardioprotection Consortium (ECC)) for multi-center randomized placebo-controlled testing of novel cardioprotective therapies in small/large animal AMI/HF models, and in AMI/HF patients, in order to improve the rigor of pre-clinical and clinical testing of novel cardioprotective therapies. In summary, the overall objective of the EU-CARDIOPROTECTION COST Action CA16225 will be to improve the translation of novel cardioprotective therapies into the clinical setting for patient benefit.

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Acute myocardial infarction (AMI) and the heart failure (HF) that complicates it are the leading causes of death and disability in Europe and worldwide. The most effective treatment for limiting myocardial infarct (MI) size and preventing HF following AMI is timely myocardial reperfusion using primary percutaneous coronary intervention (PPCI). However, despite timely PPCI, mortality and morbidity following AMI remain significant, with 7% death and 22% hospitalization for HF at one year (Cung et al., 2015). Accordingly, new treatments are required to reduce MI size, in order to preserve left ventricular (LV) systolic function and prevent the development of post-AMI HF – a treatment strategy termed "cardioprotection."

After myocardial reperfusion, the most powerful intervention for reducing MI size in the experimental setting is ischemic conditioning, an endogenous cardioprotective phenomenon by which brief episodes of ischemia and reperfusion applied to the heart or a remote organ/tissue limit MI size (Hausenloy, 2013;Hausenloy and Yellon, 2016;Hausenloy et al., 2017b).

Intensive investigation of ischemic conditioning over the last 30 years (Hausenloy et al., 2016) has identified a large number of signaling pathways and therapeutic targets for cardioprotection (Hausenloy et al., 2013;Hausenloy et al., 2017a;Hausenloy et al., 2017b). However, despite this, no effective therapies for protecting the heart against acute ischemia/reperfusion injury (IRI) have been translated into the clinical setting for patient benefit. A large number of cardioprotective therapies have been investigated in AMI patients treated with PPCI including a growing number of high-profile clinical studies, but the vast majority of these have failed to show benefit in terms of reducing MI size and improving clinical outcomes.

The **main challenge**, therefore, is to improve the translation of novel cardioprotective therapies shown to be effective in the experimental setting into the clinical arena for patient benefit. Our EU-CARDIOPROTECTION COST Action (CA16225, 4 years' duration Oct 2017 to Sept 2021) will address this challenge by setting up a pan-European network of research

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centers tasked with several key objectives, each of which will be addressed by four Working Groups (see **Figure 1**): (1) discovery of new therapeutic targets and innovative strategies for cardioprotection; (2) testing effects of combination therapy to target multiple signaling pathways both within and outside the cardiomyocyte; (3) investigating the effects of confounders (co-morbidities and co-medications) on cardioprotection; and (4) multi-center randomized controlled pre-clinical and clinical testing of novel cardioprotective therapies.

The reasons for the failure to translate novel cardioprotective therapies into the clinical setting for patient benefit are multiple and complex and have been reviewed in a number of recent papers (Ovize et al., 2010;Hausenloy et al., 2013;Lecour et al., 2014;Ibanez et al., 2015;Perrino et al., 2017;Hausenloy et al., 2017b;Heusch, 2017). Here we focus on several of the most important factors and outline how our EU-CARDIOPROTECTION COST Action will address these issues.

Therapeutic targets for cardioprotection: The majority of experimental studies have focused on targeting well-established signaling pathways and targets many of which have not proven to be beneficial in the clinical setting. Furthermore, the experimental approach has relied on a reductionist strategy focused on a single signaling pathway or target. As such, novel therapeutic targets and strategies need to be discovered in order to improve the translation of cardioprotection into the clinical setting, e.g. by taking into account unbiased "fishing" approaches by multi-omics technologies (Perrino et al., 2017).

To address this, the aim of Working Group 1 (NEW TARGETS) of our EU-CARDIOPROTECTION COST Action will be to discover novel signaling pathways and therapeutic targets within and outside the cardiomyocyte, and identify innovative strategies for cardioprotection.

Single-targeted cardioprotective therapies: The majority of experimental studies have used a single-targeted approach, directed to one signaling pathway or target within cardiomyocytes. However, a large number of different cardioprotective pathways underlie ischemic conditioning. Furthermore, the detrimental effects of acute IRI on the heart are complex, interdependent, and involve a number of players outside of the cardiomyocyte such as the coronary microvasculature, inflammatory cells, red blood cells, platelets, and extracellular vesicles (Sluijter et al., 2018;Hausenloy et al., 2017b;Heusch, 2016).

As such, the aim of Working Group 2 (COMBINATION THERAPY) of our EU-CARDIOPROTECTION COST Action will be to investigate combination therapy directed to multiple cardioprotective pathways and targets both within and outside the cardiomyocyte as an innovative approach to cardioprotection.

Experimental models with low translational value: The majority of experimental studies have used healthy juvenile animal MI models, which do not adequately reflect the clinical setting, given that most ischemic heart disease patients are middle-aged, have co-morbidities (such as diabetes, hyperlipidemia, hypertension), and are on co-medications (such as anti-platelet therapies, statins, beta-blockers, angiotensin converting enzyme inhibitors, and/or anesthetics). There are also obvious species differences in cardioprotective signaling between rodents, larger mammals and humans. These factors have been shown to confound the efficacy of cardioprotective therapies, and should be taken into consideration when evaluating novel cardioprotective therapies in experimental and clinical settings (Ferdinandy et al., 2014).

As such, the aim of Working Group 3 (CONFOUNDERS) of our EU-CARDIOPROTECTION COST Action will be to identify the key co-morbidities and co-medications which confound cardioprotection, in order to improve the translational value of the animal MI models.

Therapies with inconsistent cardioprotective efficacy: Many of the cardioprotective therapies that have failed in the clinical setting did not show consistent and robust cardioprotection in the experimental setting. This can be attributed to methodological limitations of the pre-clinical studies including lack of randomization, non-blinded treatment allocation, non-blinded data analysis, lack of standardized animal AMI/HF models and acute IRI protocols, and the lack of rigor in statistical methods. Therefore, more rigorous testing of novel cardioprotective therapies in the experimental setting are required to ensure that only the most promising therapies are investigated in the clinical arena.

To address this, the aim of Working Group 4 (CONSORTIUM) of our EU-CARDIOPROTECTION COST Action will be to set up a European Cardioprotection Consortium (ECC) for multi-center experimental testing of new cardioprotective therapies in clinically relevant small/large animals, and for testing new cardioprotective therapies in proof-of-concept clinical studies in patients subjected to acute IRI including ST-segment elevation myocardial infarction (STEMI) and coronary artery bypass graft (CABG) patients.

Research Objectives of the COST Action

Working Group 1: New Targets

The majority of the experimental studies investigating novel cardioprotective therapies have focused on targeting well-established signaling pathways/targets, many of which have not proven to be beneficial in the clinical setting. In some respects, this may have been because the therapy had not been optimized in terms of the experimental setting (dose, acute versus chronic administration and timing of therapy), and in this regard, there is an opportunity to optimize the treatment approach. In other cases, in which treatments have failed, novel therapeutic targets and strategies need to be discovered in order to improve the translation of cardioprotection from the laboratory to the clinical setting. These new cardioprotective strategies should focus on (a) discovering new therapeutic targets in novel cardioprotective pathways within the cardiomyocyte and (b) other components of acute IRI outside of the cardiomyocyte such as the microvasculature, inflammatory cells, and platelets. Potential novel targets for cardioprotection include i) inflammation targets such as macrophages/lymphocytes, RNA/DNA, and inflammasome; ii) novel mechanisms of cell death such as necroptosis and pyroptosis; iii) extracellular matrix; iv) fibroblasts; v) endothelial cells and vascular smooth muscle; vi) platelets and vii) novel mitochondrial targets(Hausenloy et al., 2017b); and viii) novel insulin targets and zinc-transporters targets associated with sarcolemma, sarco(endo)plasmic reticulum and mitochondria in hyperglycemic, failing and aging heart (Tuncay et al., 2017;Tuncay et al., 2018;Olgar et al., 2018).

The use of innovative strategies such as multi-omics (transcriptomics, epigenetics, proteomics, and metabolomics) and innovative in silico network biology evaluation is another objective of our COST Action in order to identify new therapeutic targets for cardioprotection. The pathophysiology of the ischemic heart is very complex; therefore, large-scale, unbiased, global approaches capable of identifying multiple branches of the signaling networks activated in the ischemic/reperfused heart might be a preferred approach in the discovery of novel targets. Proteomics and metabolomics offer simultaneous readouts of hundreds of proteins and metabolites altered in the ischemic myocardium, and the possibility of integrating different -omics approaches gives new hope for a

better understanding of the complicated molecular signaling activated by acute myocardial ischemia and reperfusion (Perrino et al., 2017;Varga et al., 2015). In general, WG1 will contribute to an improved knowledge of the sequence of molecular events triggered by ischemia-reperfusion that ultimately lead to tissue damage. This is required for the development of novel and effective therapeutic approaches. The identification of novel therapeutic targets may lead to the design, synthesis, and testing of novel chemical modulators in both cells and animals.

Expected Research Outputs of WG1

1. To identify at least 6 novel targets for cardioprotection either within the cardiomyocyte (newly discovered signaling pathways) or outside the cardiomyocyte (involving inflammatory cells, fibroblasts, the microvasculature, red blood cells, or platelets).
2. To identify at least 3 innovative strategies for identifying novel targets for cardioprotection such as epigenetics or multi-omics strategies (genomics, transcriptomics, metabolomics, lipidomics or proteomics).

Working Group 2: Combination Therapy

The majority of experimental studies have used a single-targeted approach, directed to a single signaling pathway and target within the cardiomyocyte. However, there exists a number of different cardioprotective pathways underlie ischemic conditioning. Furthermore, the detrimental effects of acute IRI on the heart are complex, interdependent, and involve a number of players outside of the cardiomyocyte such as the microvasculature, extracellular matrix, inflammatory cells, red blood cells and platelets. For example, it has been shown that multiple cardioprotective interventions (each with a different mechanism of action), – e.g., mild hypothermia, sodium/hydrogen exchange blocker plus a P2Y12 inhibitor, – reduced MI size to a greater extent beyond the one that achieved by a P2Y12 inhibitor alone (Yang et al., 2013b;Yang et al., 2012;Yang et al., 2013a;Yang et al., 2013c). Here we will utilize the joint expertise of different European network members to investigate combination therapy directed to multiple cardioprotective pathways and targets both within and outside the cardiomyocyte as an innovative treatment strategy for cardioprotection.

Expected Research Outputs of WG2

To identify at least 6 promising treatment strategies that are likely to have synergistic effects when administered in combination and that are directed to targets within the cardiomyocyte (newly discovered signaling pathways) or outside the cardiomyocyte (involving inflammatory cells, the extracellular matrix, the microvasculature, red blood cells, or platelets) for testing in pre-clinical acute myocardial IRI models.

Working Group 3: Confounders

Ischemic heart disease in humans is a complex disorder caused by, or associated with, cardiovascular risk factors and comorbidities, including hypertension, obesity, hyperlipidemia, diabetes, insulin resistance, heart failure, altered coronary circulation, and aging. These risk factors induce fundamental alterations in cellular signaling cascades that affect the development of IRI per se and responses to cardioprotective interventions. Moreover, some of the medications used to treat these risk factors, including statins, nitrates, and antidiabetic drugs, may impact cardioprotection by modifying cellular signaling (Ferdinandy et al., 2014).

The majority of experimental studies have used healthy juvenile animal MI models, which are of low translational value since they do not adequately reflect the clinical setting given that most CVD patients are middle-aged, have multiple co-morbidities and are on a variety of different co-medications. There are also obvious species differences in cardioprotective signaling between rodents, larger mammals, and humans. These factors have been shown to confound the efficacy of cardioprotective therapies and need to be taken into consideration when evaluating novel cardioprotective therapies in the experimental and clinical setting (Ferdinandy et al., 2014). Therefore, in this WG, we will utilize the joint expertise of the European network members to investigate key co-morbidities and co-medications that confound cardioprotection in order to improve the translational value of animal MI models.

Our aim will be to improve the pre-clinical evaluation of novel cardioprotective therapies identified in the experimental setting by investigating the effect of confounding factors such as co-morbidities (age, diabetes, hypertension, hyperlipidemia) and co-medications (anti-platelet agents, statins, beta blockers, anesthetics) on their cardioprotective efficacy. Importantly, the mechanisms through which these confounding factors interfere with cardioprotection elicited by ischemic conditioning are not clear and will also be investigated in this objective, as this will provide mechanistic insights into cardioprotection.

In order to achieve this goal, we will use more clinically relevant animal models that take into account co-morbidities and co-medications relevant to MI patients. Although studies have reported co-morbidities such as age, diabetes and left ventricular hypertrophy to attenuate the efficacy of cardioprotective therapies (Ferdinandy et al., 2014), the mechanisms underlying this effect are unknown and will be investigated in this objective. In addition, further studies are required to investigate the effect of co-medication such as anti-platelet agents, statins, anesthetics and their impact on the efficacy of new cardioprotective therapies.

Expected Research Outputs of WG3

1. To identify at least the 2 most important co-morbidities to consider when designing preclinical and clinical cardioprotection studies (factors include age, male sex, diabetes, hypertension, hypercholesterolemia).
2. To identify at least the 2 most important co-medications to consider when designing preclinical and clinical cardioprotection studies (co-medications include anti-platelet agents, statins, anesthetics, nitrates).

Working Group 4: Consortium

Many of the cardioprotective therapies that have failed in the clinical setting did not show consistent and robust cardioprotection in the experimental setting. This can be attributed to methodological limitations of the pre-clinical studies including lack of randomization, non-blinded treatment allocation, non-blinded data analysis, lack of standardized animal models and IRI protocols, and the lack of rigor in statistical methods. Therefore, more rigorous testing of novel cardioprotective therapies in the experimental setting is required to ensure that only the most promising therapies are investigated in the clinical arena. As such in this COST Action proposal, we will put in place a Consortium for (a) multi-center experimental testing of new cardioprotective therapies in clinically relevant small/large animal and human models of acute IRI; and (b) testing of new cardioprotective therapies in proof-of-concept clinical studies in patients subjected to acute IRI including STEMI and CABG patients.

The Consortium, which will be termed the European Cardioprotection Consortium (ECC), will allow multi-site testing of novel cardioprotective therapies in clinically relevant experimental small and large animal models and is, in part, modeled on the National Institute of Health-sponsored CAESAR collaborative network (Consortium for Preclinical Assessment of Cardioprotective Therapies) in the United States(Schwartz-

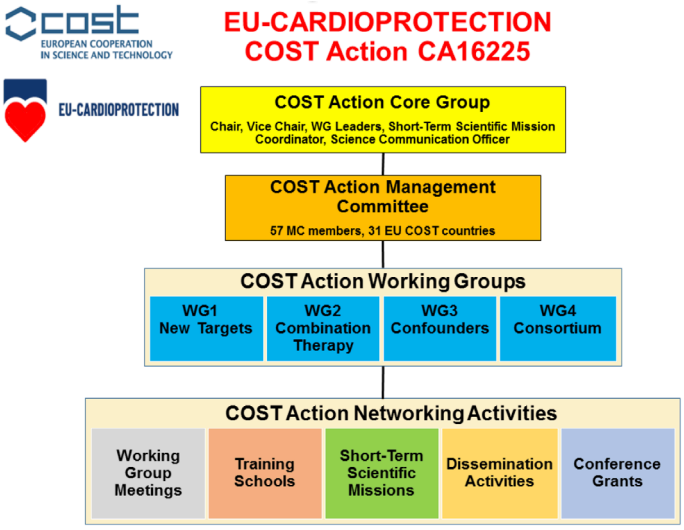

Figure 1. Overview of the EU-CARDIOPROTECTION COST Action CA16225 organization and activities. The COST Action Core Group will oversee the EU-CARDIOPROTECTION COST Action Program (4 years' duration, Oct 2017 to Sept 2021) in conjunction with the COST Action Management Committee (MC). The COST Action will utilize the COST Action Networking Activities to achieve the research objectives of the four Working Groups (WGs).

Longacre et al., 2011;Lefer and Bolli, 2011;Jones et al., 2015). The ECC will improve upon the NIH CAESAR collaborative network by (a) building on the infrastructure and knowledge obtained from the 3 WGs, in identifying novel cardioprotective targets and innovative strategies; and (b) undertaking proof-of-concept clinical studies in STEMI and CABG patients.

Expected Research Outputs of WG4

1. To put in place a network of research centers across Europe that will constitute the ECC for multi-center randomized controlled pre-clinical and clinical testing of novel cardioprotective therapies.
2. To standardize acute myocardial IRI protocols across the consortium and select which small animal (mouse, rat, rabbit) and large animal (pig, dog) AMI/HF models to include in the ECC.
3. To identify at least 4 novel cardioprotective therapies from WG 1, 2, 3 and key confounders highlighted in WG3 for testing in our ECC.
4. To identify at least 2 novel cardioprotective therapies which have demonstrated consistent and robust cardioprotection in the ECC, for future testing in proof-of-concept clinical studies in STEMI and CABG patients.

Expected impact of the EU-CARDIOPROTECTION COST Action

The immediate impact of the current project will be to create a European network of research centers to discover and disseminate novel therapeutic targets and to define strategies for cardioprotection to treat patients with co-morbidities and on co-medications. This project will establish networks and relationships with research users. The EU-Cardioprotection project involves users at all stages of research, including working with user stakeholder and participatory groups. The short-term impact will be achieved by exchanging ideas, knowledge, tools, and data of scientists working on different but related systems using various approaches.

The short-term scientific, technological and socio-economic impacts will provide: i) The knowledge basis for research scientists at all levels and industrial players to increase the number of successful innovative development projects aimed at discovering novel cardioprotective therapies. The COST Action will provide state-of-the-art, detailed protocols for measuring MI size in mice, rabbits, and pigs in a manner that is rigorous, accurate, and reproducible, thereby helping to best inform which therapies should be taken forward by industrial partners for further development and commercialization. **ii)** The capability of the COST Action members to jointly apply for a Horizon 2020 grant to set up the European Cardioprotection Collaboration network of research centers for the pre-clinical and clinical testing of novel cardioprotective therapies as outlined in WG4 of the project. **iii)** The ability to validate the efficacy and safety of current cardioprotective strategies such as ischemic postconditioning and remote ischemic conditioning in the treatment of AMI in the real world, i.e. in patients with major co-morbidities and co-medications. **v)** Data for early exclusion of candidate strategies unlikely to succeed in AMI patients.

The long-term scientific, technological and socio-economic impacts include: i) accumulation of the critical mass to understand problems with high levels of complexity such as cardioprotection -- problems that can only be solved by a concerted effort of multidisciplinary teams; **ii)** enabling researchers from less research intensive countries to interact and share ideas, resources and personnel with scientists from countries in which research enjoys a high priority **iii)** creation of new, and strengthening of existing, interactions among European scientists and international experts via our external advisory board; **iv)** setting up the ECC network of research centers for pre-clinical and clinical testing of novel cardioprotective therapies, which will lead to successful development of cardioprotective therapies, thereby solving an unmet need in the treatment of ischemic heart disease, which is currently the leading cause of morbidity and mortality in the EU; **v)** increasing the number and success rate of innovative development projects, creation of new jobs and increased competitiveness of innovative small-to-medium enterprises (SMEs); **vi)** heightening the impact of cardioprotection research on policy makers, regulatory bodies and national decision makers as well as on the private sector; **vii)** developing novel technologies and services that will impact on the growth of the EU's knowledge-driven economy; and **viii)** contributing to healthy aging in the European population and providing a major impact on quality of life.

COST Action research network activities

The following Network Activities listed below will be used in our EU-CARDIOPROTECTION COST Action to deliver the main research objectives of our 4 WGs (see Figure 1).

WG Meetings: Bi-annual WG Meetings will allow the COST partners and others to disseminate and share knowledge relevant to the objectives of the 4 different, but closely inter-related, WGs. Invited speakers and chairs will be considered with respect to gender balance and geographical distribution. Early career investigators (ECIs) will be encouraged to chair sessions and present at the WG Meetings. We will invite leading international experts in preclinical and clinical cardioprotection to attend the meeting and provide expert input and advice to the relevant WGs. We will also invite international researchers who have expertise in setting up a similar cardioprotection research consortium in the U.S. – the NIH CAESAR Network -- to provide advice on setting up our ECC in WG4. Our first WG Meeting took place in October 2017 in Brussels at the COST Headquarters, and outlined the main objectives and activities of our EU-CARDIOPROTECTION Cost Actions. Our next WG meeting, which will be held in the Medical University of Vienna, Austria, in March 2018 (local organizers, Bruno Podesser and Mariann Gyöngyösi), will begin the implementation of strategies to achieve the WG objectives of the COST Action.

Training schools: In order to support the training and development of ECIs, training schools will be organized on a variety of topics relevant to cardioprotection research including (1) novel methods for discovering novel targets for cardioprotection and innovative cardioprotective strategies such as multi-omics approaches; (2) the use of combination therapies as a multi-targeted approach to cardioprotection; (3) clinically relevant animal AMI/HF based on co-morbidities (age, diabetes, hypercholesterolemia) and consideration of relevant co-medications (statins, nitrates, anesthetics, anti-platelet agents); and (4) guidelines for undertaking multicenter experimental studies and proof-of-concept clinical studies.

Short-term Scientific Missions (STSMs): In order to achieve long-lasting effects of this COST Action, ECIs will be supported in their networking activities by STSMs (duration of one week to 3 months and supported by a fixed grant from the COST Action). These will allow ECIs to learn new research techniques and gain valuable knowledge and experience from an institution or laboratory in another COST country. Increasing collaboration is important to make Europe more competitive. Exchanging ideas and knowledge across borders will lead to more successful projects. So far our EU-CARDIOPROTECTION COST Action has initiated 4 STSMs involving COST Member Countries and International Partner Countries (UK-Singapore, Greece-Germany, Slovakia-Hungary, Romania-Austria). We intend to initiate a further 8 STSMs each year for the duration of the COST Action.

Dissemination activities: A website providing access to the generated knowledge and annual newsletters, with mutual links to websites relevant to this COST Action has been set up at www.cardioprotection.eu. Scientific publications disseminating knowledge of the WG will be published as original articles, reviews and position papers in conjunction with other cardiovascular organizations and societies. Our COST Action will also sponsor joint scientific sessions at other European and international cardiovascular meetings including the International Society of Heart Research-European Section (ISHR-ES) meetings and ESC Working Group on Cellular Biology of the Heart.

Conference Grants: This will enable PhD students and ECIs from participating Inclusiveness Target Countries to attend international cardiovascular conferences that are not organized by our COST Action.

Summary of the EU-CARDIOPROTECTION COST Action

In summary, our COST Action will put in place a pan-European Research Network of leading experts in cardioprotection to jointly develop new initiatives and new strategies for finding innovative and more effective approaches to cardioprotection and for optimizing the pre-clinical and clinical evaluation of new cardioprotective therapies, so as to improve their translation into the clinical setting for patient benefit. The COST Action will co-ordinate and strengthen European research in the field of cardioprotection and accelerate scientific progress through the dissemination and sharing of new therapeutic targets among network members and industrial partners, thereby facilitating the discovery of new cardioprotective therapies. By utilizing the joint expertise of different European network members, we will investigate factors that confound the efficacy of new cardioprotective therapies, including comorbidities (such as age, diabetes, and hypertension) and co-medications (such as anti-platelet therapies, statins and beta-blockers). Finally, we will set up a European network of research centers for multi-center laboratory testing of new cardioprotective therapies using small and large animal models of acute IRI in order to select those therapies most likely to succeed in the clinical setting. All aspects of this COST Action proposal require a critical mass of partners covering a wide geographic distribution across Europe in order to deliver the objectives outlined in this proposal and improve clinical outcomes for AMI patients in Europe and worldwide.

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Conflict of interest

HEB is shareholder in CellAegis Inc. PF is the founder and CEO of Pharmahungary Group, a group of R&D companies focusing on services and novel technologies for cardioprotection and its comorbidities (www.pharmahungary.com). DES declares speaker fees from ZOLL. FP received research grants from Bayer and Boehringer. All other authors declare no conflict of interest.

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