Is Cardioprotection Dead?

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Abstract

For >4 decades, the holy grail in the treatment of acute myocardial infarction has been the mitigation of lethal injury. Despite promising initial results and decades of investigation by the cardiology research community, the only treatment with proven efficacy is early reperfusion of the occluded coronary artery. The remarkable record of failure has led us and others to wonder if cardioprotection is dead. The path to translation, like the ascent to Everest, is certainly littered with corpses. We do, however, highlight a therapeutic principle that provides a glimmer of hope: cellular postconditioning. Administration of cardiosphere-derived cells after reperfusion limits infarct size measured acutely, while providing long-term structural and functional benefits. The recognition that cell therapy may be cardioprotective, and not just regenerative, merits further exploration before we abandon the pursuit entirely.

Keywords
cardiosphere derived cells; cell- and tissue-based therapy; heart failure; myocardial Infarction; ventricular function, left

Since 1937, when Gross et al\textsuperscript{1} first attempted to reduce myocardial infarct size (by coronary sinus occlusion), many cardioprotective strategies have been devised and tested. Percutaneous coronary intervention is the only such strategy to have withstood the test of time: it has become standard therapy for patients with acute myocardial infarction (AMI), not only reducing immediate mortality and morbidity, but also improving long-term outcomes.\textsuperscript{2} Nevertheless, some AMI patients, particularly those who end up with a large infarct size, progress to heart failure even with best current therapy. Adverse left ventricular (LV) remodeling after AMI is a precursor to the development of overt heart failure and heralds increased mortality.\textsuperscript{3,4} In an effort to avert heart failure post–myocardial infarction (MI), numerous adjunctive strategies to reduce infarct size have been tested (>6400 articles on cardioprotection published since 1975). Many have been founded on well-reasoned pathophysiological hypotheses accepted by entire communities of investigators, but, aside

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from prompt reperfusion, nothing, not one drug or product of potential clinical utility, has emerged. This failure to translate is not attributable to a lack of effort or resources: in the United States alone, the quest for acute cardioprotective therapies has involved a collective investment of several hundred million dollars from the National Heart, Lung, and Blood Institute. Much important work in this area has been done outside the United States, so that the failed expenditure worldwide likely totals ≥$1 billion just on the academic side. Here, we review the dismal history of adjunctive approaches to limit infarct size. Is cardioprotection dead? Or are there new, viable approaches that have the potential to resuscitate this moribund concept?

Sorting the wheat from the chaff is a nontrivial matter when it comes to infarct size–limiting agents. In an effort to validate therapeutic candidates, in 2011 the National Heart, Lung, and Blood Institute established a network called the Consortium for Preclinical Assessment of Cardioprotective Therapies (CAESAR) that was based on the principles of clinical trials (ie, randomization, investigator blinding, exclusion criteria, and appropriate statistical analyses). A number of putatively cardioprotective agents were tested in the CAESAR network, but none was found to be effective under these rigorous experimental conditions. Table 1 summarizes failures to confirm once-promising preclinical data, including those debunked by CAESAR and publicly reported (many others failed in CAESAR but results have not yet been published). Independent of CAESAR, a number of drugs have been tested in patients, with similarly dispiriting results (Table 2). In particular, once the tissue has been reperfused, nothing seems to work. Patients can rarely predict when they will have an AMI, and doctors similarly lack clairvoyance. Ischemic postconditioning (created by cyclic intracoronary balloon inflations) requires immediate manipulation of flow at the time of reperfusion, with loss of benefit if there is delay.\textsuperscript{29,30} A key consideration of any adjunctive therapy is compatibility with standard clinical practice: in assessing new therapies, it is important to devise interventions that work even after the occluded artery has been successfully opened.

Despite the disappointments to date, a new direction has arisen from an unlikely corner: cell therapy. The conventional rationale for cell therapy is to trigger regeneration, not cardioprotection. The dogma is as follows: progenitor cells, if transplanted into the postischemic heart, will implant, proliferate, and differentiate into viable myocardium. Healing occurs by the growth of new, healthy heart muscle, not by preservation of at-risk myocardium. In such a paradigm, cardioprotection plays no role. Recently, we and others have discovered that cell therapy may indeed be effective in limiting injury when given shortly after AMI, but the protection does not require long-term cell implantation, nor does it involve canonical stem cell mechanisms. Instead, transplanted cells recruit cardioprotection. The collective evidence, reviewed here, gives reason to hope that cardioprotection may not be entirely dead after all…perhaps just stunned.

**CELL THERAPY FOR AMI**

Numerous lines of evidence now support the idea that cells can either be cardioprotective (when administered during or soon after AMI) or regenerative (when administered after scar is well established). Work in a bitransgenic fate-mapping mouse model revealed that the 2 mechanisms are not mutually exclusive: they contribute roughly equally to the long-term (3
weeks post-MI) increase in myocardial viability when cells are given shortly after permanent coronary ligation. Three-week end points or longer will lump both contributions together; short-term end points (e.g., 48 hours) enable study of the cardioprotective effect in isolation, well before the regenerative mechanisms of cardiomyocyte proliferation and activation of endogenous cardioblasts come into play (on a time scale of weeks). Many animal studies have investigated cells in nonreperfused AMI, and several others have targeted chronically scarred myocardium. Surprisingly little is known about the utility and risks of intra-coronary cell administration soon after (i.e., within 20–45 minutes of) reperfusion. No clinical data are available; cell therapy clinical trials have generally infused cells 1 to 14 days post-AMI. By that time, cardiomyocytes at risk are already dead, so there is limited potential (if any) for myocardial salvage. Given the delays intrinsic to autologous tissue harvesting and cell processing, applications in the acute reperfusion phase will require allo- geneic (off-the-shelf donor-derived) products. Preclinical studies of acutely administered allogeneic mesenchymal stem cells or their precursors have yielded variable results. Some studies have questioned the safety of intra-coronary infusion of cells post-MI, with decreased coronary flow and elevation of cardiac enzymes attributed to microvascular plugging. Houtgraaf et al had more favorable results with mesenchymal precursor cells after careful attention to cell dosage and infusion rate. These investigators began infusion at 15 minutes of re-flow, and they quantified infarct size only at 8 weeks, at which time longer-term regenerative effects may cloud the evaluation of cardioprotection.

Table 3 lists all cell types that have been tested in animal models of AMI, along with the following information for each cell type: the most advanced preclinical model tested; the immune match tested (syngeneic, allogeneic, and xenogeneic); whether or not cardioprotection has been demonstrated histologically in 48- to 72-hour follow-up after postreperfusion cell delivery; and clinical testing status (any clinical testing, and clinical testing specifically in AMI adjunctive to reperfusion). The only cell types to have been shown to be cardioprotective are cardiosphere-derived cells, which are discussed further below.

CARDIOSPHERE-DERIVED CELLS

Over the past 12 years, cardiosphere-derived cells (CDCs) have emerged as a candidate cell type for regenerative therapy post-MI. Unlike many other cell therapy products, the mechanism of action of CDCs is well understood. These heart-derived cells exhibit multilineage potential and clonogenicity, but they work primarily through indirect mechanisms. At least 35 independent laboratories worldwide have generated CDCs and verified their therapeutic bioactivity. CDCs were first tested clinically in the CADUCEUS trial (Cardiosphere-Derived Autologous Stem Cells to Reverse Ventricular Dysfunction), which examined the safety and efficacy of intracoronary autologous CDCs in 17 patients with LV dysfunction and convalescent MI (1.5–3 months prior), in comparison with 8 randomly assigned controls. The results were promising in revealing evidence of therapeutic regeneration with CDCs, but the chronicity of the MI ruled out any contribution from cardioprotection in that study. Although heart-derived stem cells have been tested in both large animals and humans in chronic ischemic settings until recently, the only studies using an acute ischemia/reperfusion (I/R) model were in rats, where structural and functional outcomes were improved dramatically by the intracoronary infusion of
allogeneic CDCs 20 minutes post-AMI. However, the 3-week end point in those studies made it impossible to separate cardioprotection from regeneration.

**INDIRECT EFFECTS OF CDCS**

In the vast majority of experimental studies, the number of differentiated myocytes derived from transplanted stem cells is too small to account for the observed improvements in cardiac function. Thus, the prevailing concept of stem cell efficacy has shifted toward the paracrine hypothesis, which proposes that transplanted cells produce soluble factors beneficial to the infarcted heart. Potential cardioproteective effects of paracrine factors include antiamoapoptotic effects on resident myocytes, upregulation of angiogenesis, modulation of inflammatory processes resulting in better infarct healing, promotion of cardiomyocyte cell cycle reentry, and induction of secondary humoral effects in the host tissue. Recent findings implicate exosomes as critical agents of the indirect effects of CDCs, likely attributable, at least in part, to the transfer of cardio-protective and regenerative microRNAs (eg, miR-146a) from CDCs to surrounding heart tissue.

**ROLE OF INFLAMMATION AND MACROPHAGES**

Innate immunity pathways are recruited to deal with sterile inflammation, as occurs in AMI. The first step is an intense influx of neutrophils within minutes of injury. Macrophages (Mφ) are then mobilized to clear necrotic debris, antagonize further neutrophil entry, and begin wound healing. Although there is ample evidence that neutrophils exacerbate I/R injury by killing damaged (but salvageable) cardiomyocytes, nonselective inhibition of inflammation has not proven to be useful therapeutically. Targeting of distinct immune cell populations and subpopulations may be a more viable strategy. Mφ, in particular, are an important potential target; they can originate within the heart (tissue-resident Mφ) or from a blood-borne influx of monocytes, which then differentiate into Mφ in the tissue. Despite the common classification of Mφ into either M1 or M2 subpopulations (with proinflammatory or reparative properties, respectively), Mφ are highly plastic and can assume a variety of activated states in response to microenvironmental cues. In fact, at least 4 distinct resident Mφ subsets exist within the adult heart under normal conditions. Following AMI, both resident and monocyte-derived Mφ expand their populations to regulate repair with several distinct phenotypes, modulating phagocytosis, antigen presentation, and T-cell activation. It is interesting to note that in the neonatal heart, Mφ are essential for cardiac regeneration, a function lost within days of birth.

**CELLULAR POSTCONDITIONING**

In 2014, the laboratory of one of the authors (E.M.) demonstrated the phenomenon of cellular postconditioning: CDCs are cardioprotective when given within a reasonable delay after I/R in AMI. In pigs subjected to 90 minutes of ischemia and 30 minutes of reflow, the intracoronary infusion of CDCs decreased infarct size and also reduced the extent of microvascular occlusion measured at 48 hours. Cyclic sham interruptions of coronary flow starting 30 minutes post-I/R were not cardioprotective, distinguishing CDC-related cardioprotection from ischemic postconditioning. To be absolutely certain that ischemic...
postconditioning did not confound the results, we performed a new set of experiments using nonocclusive continuous-flow methods to deliver CDCs into the infarct-related artery 30 minutes after reflow in AMI pigs. Figure 1 confirms robust infarct size reduction measured histologically, and preservation of LV ejection fraction, wall thickness, and wall motion using MRI, as well. These new data provide additional evidence of the protective effects of CDC postconditioning. After the initial report in pigs, we published a follow-on mechanistic study in rats with AMI. This work confirmed and extended the initial findings: intracoronary infusion of CDCs at 20 minutes of reperfusion reduced infarct size and improved functional recovery. CDCs decreased the number of myocardial CD68+ Mϕ, and these CDCs secreted factors that polarized Mϕ toward a distinctive cardio-protective phenotype. Systemic depletion of Mϕ with clodronate abolished CDC-mediated cardioprotection. Post-I/R adoptive transfer of CDC-conditioned Mϕ also reduced infarct size, recapitulating cellular postconditioning. Thus, CDCs appear to limit acute injury by polarizing an effector Mϕ population within the heart.

Given the concerns articulated earlier, any putative new cardioprotective mechanism will understandably be greeted skeptically. Thus, independent validation of the basic phenomena is highly desirable. Using blinded analysis and randomization, the Lefer laboratory (one of the principals in the CAESAR network, and an author here) has now independently reproduced the findings of robust cardioprotection by CDCs. Figure 2 shows the results of a study in which allogeneic rat CDCs were administered 20 minutes following reperfusion in the spontaneously hypertensive rat AMI model. Placebo (phosphate-buffered saline)–injected spontaneously hypertensive rats exhibit very large areas of infarction (ie, >50% of the area-at-risk) following coronary I/R. CDC postconditioning significantly attenuated myocardial infarct size and plasma cardiac troponin I levels at 48 hours postreperfusion. It is interesting to note that LV structure and function were preserved in CDC-treated spontaneously hypertensive rats at 28 days post-AMI in comparison with rats that had received phosphate-buffered saline (Figure 2), verifying that the effects are durable (as the Marbán laboratory had shown in another pig study).

Figure 3 summarizes our current understanding of the mechanisms of CDC-mediated postconditioning. Extensive evidence supports the notion that extracellular nanovesicles called exosomes are secreted by CDCs and mediate their salient effects, likely via cell-cell transfer of noncoding RNAs, including microRNAs (although exosomes contain a redundancy of other bio-active molecules, including proteins and transcripts). The effects of CDCs on macrophages are replicated by CDC-secreted exosomes (CDCexo), and CDCexo themselves mimic cellular postconditioning. Although the cascade of microRNA transfer and target gene suppression might seem too slow to mediate a process that necessarily must be rapid to prevent substantial cardiomyocyte death, microRNAs are known to be capable of suppressing proinflammatory gene expression in just 1 hour. A host of acute and longer-term salutary effects ensue. Within just 2 hours, cardiomyocyte apoptosis in the postischemic heart is inhibited by ≈60%. Meanwhile, macrophages are altered so as to become cytoprotective. CDCexo–Polarized macrophages exhibit enhanced phagocytosis; our working hypothesis posits that such macrophages become more efficient in clearing necrotic debris (thereby enhancing the healing process known as efferocytosis). The net effects are a reduction of infarct size evident early, with sustained structural and functional benefits.
The recognition of a central mechanistic role for CDC_{exo} begs the question of whether cell-free therapeutics may be able to recruit benefits equivalent to cellular postconditioning. In the long run, this possibility seems likely: as we come to recognize the key bioactive components within CDC_{exo}, they may become effective therapeutic agents on their own, either naked or packaged within designer exosomes. In the immediate future, however, CDC_{exo} themselves may not be a realistic, alternative therapeutic candidate to CDCs. Although CDC_{exo} reproduce the salient benefits of CDCs, we have recently reported, in a porcine model of cellular postconditioning, that intramyocardial injection is required for efficacy.\textsuperscript{96} The intracoronary route is far preferable clinically, especially in the setting of recent reperfusion when the heart can be particularly susceptible to ventricular arrhythmias.\textsuperscript{97} Thus, CDCs, which are effective after intracoronary delivery (Figures 1 and 2, and references 55–57), continue to be the prime therapeutic candidate for reducing infarct size translationally, as discussed further below.

NEW CONCEPTS SUGGEST NEW APPROACHES

The discovery that CDCs work in AMI despite being administered with some delay after reperfusion is notable, because it avoids the need for pretreatment and immediate intervention on reopening the affected artery.\textsuperscript{98} The concept of cellular postconditioning is novel, and merits comparison with other cardioprotective processes that can be recruited pharmacologically and by transient ischemia (preconditioning and ischemic postconditioning). Unlike those phenomena, however, cellular postconditioning has the unique advantage of being recruitable 30 minutes after reperfusion (and perhaps even longer; the precise limits of the cardioprotective window remain to be defined). The idea that cell therapy may mitigate ischemic injury by modulating Mϕ is supported by recent work,\textsuperscript{89} and is consistent with the immunomodulatory properties described for CDCs.\textsuperscript{99,100} Although inflammation figures prominently in AMI, there has been little by way of targeted intervention to take advantage of our exploding knowledge of innate immunity pathways and Mϕ biology. Although not originally conceived as selectively targeting inflammation to reduce infarct size, CDCs may turn out to achieve this long-elusive goal.

PROSPECTS FOR TRANSLATION

Few of the cell types tested preclinically in AMI model have progressed to clinical testing (Table 3). As summarized in Table 4, allogeneic CDCs are already in advanced clinical testing; they have proven safe to date in >100 patients treated by coronary infusion.

Thus, from a product readiness viewpoint, it should be straightforward to initiate clinical testing of the hypothesis that CDCs induce cellular postconditioning, targeting end points including infarct size and LV ejection fraction. Demonstration of efficacy in humans would comprise the ultimate proof of concept that cellular postconditioning is genuine. Nevertheless, some cautionary notes are worth considering before launching into clinical trials. First, dosing of the CDCs needs to be carefully adjusted. CDCs are large cells that can be microcclusive.\textsuperscript{34} In the setting of AMI, where microvascular occlusion already can occur, intermediate dosing may be required: too few infused cells will be ineffective, while too many may actually worsen preexisting microvascular occlusion. Even in the highly
controlled pig model, we have found that excessively high doses result in decreased efficacy, consistent with the Goldilocks caveat. In humans with highly variable degrees of I/R injury on presentation with AMI, it will be even more challenging to estimate a safe-but-effective dose. A second consideration is the fact that it is exceedingly difficult, in the AMI setting, to determine which patients will go on to develop large infarcts. The results of early percutaneous intervention are so overwhelmingly positive, even for patients presenting with hypotension and tombstone Ts, that entry criteria are now difficult to establish reliably for any cardioprotective protocol. On balance, proceeding with relatively low CDC doses and broad inclusion criteria seems most prudent, recognizing that the number of patients one must treat to see benefit will necessarily be increased by such a conservative approach. The ongoing AMICI trial (Safety Study of Allogeneic Mesenchymal Precursor Cell Infusion in Myocardial Infarction) of allogeneic mesenchymal precursor cells in AMI may provide helpful safety data and insights into dosage to help guide future trials (Clinicaltrials.gov NCT01781390). Indeed, mesenchymal precursor cells are the first cells to be tested clinically as adjunctive therapy to percutaneous intervention in AMI (Table 3).

CONCLUSIONS

Despite >40 years of effort and thousands of reports of therapies claiming to limit myocardial infarct size in the setting of AMI, there are no approved treatments to supplement the unambiguous efficacy of early reflow. Recent data reviewed here demonstrate the powerful effects of cellular postconditioning with CDCs administered following reperfusion. Here we additionally provide compelling unpublished data from 2 different laboratories in 2 different animal species demonstrating robust cardioprotection when CDCs are administered 20 to 30 minutes following reperfusion. These studies demonstrate major reductions in myocardial infarct size in the spontaneously hypertensive rat and in the Yucatan miniswine model. Reductions in infarct size are accompanied by improved LV function and preservation of myocardial blood flow with attenuated no-reflow. Cellular postconditioning may be clinically tractable, providing new hope that myocardial reperfusion injury can be effectively targeted. The jury is still out, but we conclude that cardioprotection against AMI is not dead…at least, not yet.

Acknowledgments

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References


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69. Li TS, Cheng K, Lee ST, Matsushita S, Davis D, Malliaras K, Zhang Y, Matsushita N, Smith RR, Marbán E. Cardiospheres recapitulate a niche-like microenvironment rich in stemness and cell-


Figure 1. Validation of cellular postconditioning in pigs
A. MR short-axis images from a placebo and CDC-treated pig. Transverse cardiac slices stained with Thioflavin T and Gentian Violet (B), and triphenyl tetrazolium chloride (TTC) (C) in the same representative sample. B. The area of MVO appears nonfluorescent under UV light, whereas the area-at-risk (AAR) is unstained with Gentian Violet. C. Viable myocardium appears red and scar appears white/yellow. LVEF (D, top), infarct wall thickening (D, middle), and infarct wall motion (D, lower) are improved following CDC treatment. MVO/AAR (E, top), and infarct size (IS)/AAR (E, middle) are decreased following CDC treatment, whereas AAR is not different between groups (E, lower). Graphs depict mean±SEM. Statistical significance was determined by using the Student t test.
*P<0.05. CDC indicates cardiosphere-derived cell; LVEF, left ventricular ejection fraction; MVO, microvascular occlusion; and SEM, standard error of the mean.
Figure 2. Cellular postconditioning in spontaneously hypertensive rats
A, Experimental protocol involving male spontaneously hypertensive rats (SHRs) subjected to 30 minutes of left coronary artery ischemia followed by either 48 hours (h) or 4 weeks (w) of reperfusion. Myocardial area-at-risk and infarct size were determined at 48 h postreperfusion. Plasma levels of cardiac troponin I (cTnI) was measured at 2 h and 48 h of reperfusion. At 20 minutes of reperfusion, rat CDCs (0.5×10^6) or phosphate-buffered saline (PBS) were injected directly into the left ventricular lumen following aortic cross-clamping.
B, Representative photomicrographs of SHRs receiving either PBS or CDCs at 20 minutes of reperfusion. Myocardial infarct size is significantly attenuated in the CDC-treated heart.
C, Myocardial area-at-risk (AAR) as a percentage of the left ventricle (LV), infarct size (INF) per AAR, and INF as a percent of the LV in rats receiving either PBS or CDCs. Myocardial infarct size per area-at-risk or LV was significantly (P<0.01) reduced in the CDC group. D, Plasma cardiac troponin I (cTnI) levels at 2 and 48 h following reperfusion. cTnI levels are significantly (P<0.05) reduced at 48 h postreperfusion. E, Left ventricular ejection fraction (LVEF) at baseline and at 4 weeks following reperfusion. LVEF is similar at baseline and significantly (P<0.05) greater in animals receiving CDCs. F, Left ventricular end-systolic dimension (LVESD) at baseline and 4 weeks of reperfusion in the PBS and CDC groups. LVESD is significantly (P<0.05) reduced in the CDC group in comparison with PBS. G, Interventricular septal dimension at end-systole (IVSs) at baseline and 4 weeks following reperfusion. IVSs was significantly (P<0.01) greater in hearts treated with CDCs than with PBS. H, Interventricular septal dimension at end-diastole (IVSd) at baseline and 4 weeks postreperfusion. Similar to IVSs, IVSd was significantly (P<0.01) greater in the CDC group than in the PBS group. Numbers inside the bars represent the number of animals in each group. Statistical significance was determined by using the Student t test. CDC indicates cardiosphere-derived cell; and 2,3,5-TTC, 2,3,5-triphenyltetrazolium chloride.
*Plasma samples for cTnI. ‡Myocardial infarct size analysis. †2-D Echocardiography, Visual Sonics Vevo 2100.
Figure 3. Mechanisms of CDC-mediated cellular postconditioning
Cardiosphere-derived cells (CDCs) release exosomes resulting in the transfer of RNA and proteins to macrophages, fibroblasts, endothelial cells, and cardiomyocytes, in turn, leading to both acute and late cardioprotective actions. In the acute phase of reperfusion injury, CDCs improve cardiomyocyte viability and reduce myocardial infarct size by the conversion of resident macrophages to a cardioprotective phenotype and dampening the innate immune response. During the later phases of myocardial re-perfusion injury, CDC therapy results in sustained infarct size reduction by alterations in polarization of infiltrating macrophages, accelerated clearance of necrotic debris, and significant attenuation of the late inflammatory response in the myocardium.
Table 1
Cardioprotective Agents That Have Been Tested in Preclinical Studies to Reduce Myocardial Infarct Size or Improve Left Ventricular Function and Have Failed

<table>
<thead>
<tr>
<th>Agent</th>
<th>Model and Study Authors</th>
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<tbody>
<tr>
<td>Allopurinol</td>
<td>Canine (40 min + 4 days); Reimer and Jennings (1985)(^5)</td>
</tr>
<tr>
<td>Superoxide dismutase + catalase</td>
<td>Canine (3 h + 24 h); Gallagher et al (1986)(^6)</td>
</tr>
<tr>
<td>Superoxide dismutase</td>
<td>Canine (40 min + 4 days); Uraizee et al (1987)(^7)</td>
</tr>
<tr>
<td>Oxypurinol</td>
<td>Canine; Puett et al (1987)(^8)</td>
</tr>
<tr>
<td>Anti-polymorphonuclear antibody</td>
<td>Canine (3 h + 21 h); Chatelain et al (1987)(^9)</td>
</tr>
<tr>
<td>Superoxide dismutase + catalase or oxypurinol</td>
<td>Canine (90 min + 4 days); Richard et al (1988)(^10)</td>
</tr>
<tr>
<td>Polyethylene glycol superoxide dismutase</td>
<td>Canine (90 min + 4 days); Tanaka et al (1990)(^11)</td>
</tr>
<tr>
<td>Anti-CD18 monoclonal antibody</td>
<td>Canine (90 min + 3 h); Tanaka et al (1993)(^12)</td>
</tr>
<tr>
<td>Sildenafil</td>
<td>Porcine (60 min + 48 h); Kukreja et al (2014)(^*)</td>
</tr>
<tr>
<td>Sodium nitrite</td>
<td>Porcine (60 min + 48 h); Lefer et al (2014)(^*)</td>
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For the outcome of all agents, there was no effect on infarct size.

Table 2

Potential Cardioprotective Agents That Have Been Tested in Clinical Trials to Reduce Myocardial Infarct Size or Improve Left Ventricular Function and Have Failed

<table>
<thead>
<tr>
<th>Agent</th>
<th>Study</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hyaluronidase</td>
<td>Prethrombolytic era</td>
<td>No effect on infarct size</td>
</tr>
<tr>
<td>Calcium channel blocker</td>
<td>SPRINT II; Goldbourt et al (1993)</td>
<td>Increased mortality</td>
</tr>
<tr>
<td>Free radical scavenger, human superoxide dismutase</td>
<td>Flaherty et al (1994)</td>
<td>No improvement in left ventricular function</td>
</tr>
<tr>
<td>Antioxidant-trimetazidine</td>
<td>ESPRIM; ESPRIM Group (1994)</td>
<td>No effect on mortality or clinical outcomes</td>
</tr>
<tr>
<td>Fluosol</td>
<td>TAMI-9; Wall et al (1994)</td>
<td>No decrease infarct size or increase in left ventricular function</td>
</tr>
<tr>
<td>Rheoth RX-poloxamer 188</td>
<td>EMIP-FR; EMIP FR Group (2000)</td>
<td>No effect on death, shock, or reinfarction</td>
</tr>
<tr>
<td>White blood cell inhibitor: Anti-CD18 monoclonal antibody</td>
<td>FESTIVAL; Rusnak et al (2001)</td>
<td>No decrease in infarct size</td>
</tr>
<tr>
<td>Na^+H^+ exchange inhibitor</td>
<td>ESCAMI; Zeymer et al (2001); CASTEMI; Bar et al (2006)</td>
<td>No effect on infarct size, clinical outcomes, left ventricular ejection fraction</td>
</tr>
<tr>
<td>Complement inhibitors</td>
<td>COMPLY Trial; Mahaffey et al (2003); APEX Trial; Armstrong and Granger (2007)</td>
<td>No decrease in infarct size or decrease in mortality</td>
</tr>
<tr>
<td>Magnesium</td>
<td>Magnesium in Coronaries Trial Investigators (2002)</td>
<td>No effect on mortality, heart failure, or ventricular tachycardia</td>
</tr>
<tr>
<td>Nicorandil</td>
<td>Kitakaze et al (2007)</td>
<td>No effect on mortality or infarct size</td>
</tr>
<tr>
<td>Cold perfusion</td>
<td>CHILL-MI; Erlinge et al (2014)</td>
<td>No effect on infarct size or left ventricular ejection fraction</td>
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<tr>
<td>Sodium nitrite</td>
<td>NIAMI; Siddiqi et al (2014)</td>
<td>No decrease in infarct size, no effect on left ventricular ejection fraction</td>
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<tr>
<td>MPTP inhibitor</td>
<td>MITOCARE; Atar et al (2014)</td>
<td>No effect on infarct size or left ventricular ejection fraction</td>
</tr>
<tr>
<td>Cyclosporine</td>
<td>CIRCU; Cung et al (2015)</td>
<td>No effect on deaths or heart failure</td>
</tr>
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</table>

Adapted and extended from Robert Kloner, MD, National Institutes of Health Workshop on New Horizons in Cardioprotection, 2011. APEX indicates Pexelizumab in Conjunction With Angioplasty in Acute Myocardial Infarction; CASTEMI, Caldaret in ST Elevation MI; CHILL-MI, Rapid endovascular catheter core cooling combined with cold saline as an adjunct to percutaneous coronary intervention for the treatment of acute myocardial infarction; CIRCUS, Cyclosporine and Prognosis in Acute Myocardial Infarction Patients; COMPLY, Complement inhibition in myocardial infarction treated with thrombolytics; EMIP-FR, European Myocardial Infarction Project - Free Radicals; ESCAMI, Evaluation of the safety and cardioprotective effects of emiporide in acute myocardial infarction; FESTIVAL, An anti-CD11/CD18 monoclonal antibody in patients with acute myocardial infarction having percutaneous transluminal coronary angioplasty; MITOCARE, Effect of intravenous TRO40303 as an adjunct to primary percutaneous coronary intervention for acute ST-elevation myocardial infarction; MPTP, mitochondrial permeability transition pore; NIAMI, intravenous sodium nitrite in acute ST-elevation myocardial infarction; SPRINT II, Systolic Blood Pressure Intervention Trial II; and TAMI-9, Thrombolysis and Angioplasty in Myocardial Infarction-9.

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Table 3

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>Most Advanced Model Tested</th>
<th>Immune Match? (Allo-, Syn-, or Xenogeneic)</th>
<th>Cardioprotection Demonstrated in Realistic Acute Myocardial Infarction Model?</th>
<th>Clinical Testing Status</th>
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<tr>
<td>MSCs</td>
<td>Pig</td>
<td>Allo/Syn</td>
<td>No</td>
<td>Yes</td>
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<tr>
<td>UBMNCs</td>
<td>Pig</td>
<td>Allo/Xeno</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>BMNCs</td>
<td>Pig</td>
<td>Allo/Syn</td>
<td>No</td>
<td>Yes</td>
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<tr>
<td>EPCs</td>
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<td>Allo/Syn/Xeno</td>
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<td>No</td>
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<td>BATDCs</td>
<td>Rat</td>
<td>Syn</td>
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<td>No</td>
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<tr>
<td>CD31+ BMDPCs</td>
<td>Pig</td>
<td>Syn</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>MPAPCs</td>
<td>Rodent</td>
<td>Syn/Allo</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>ESC-Derived CMs</td>
<td>Monkey</td>
<td>Xeno/Syn</td>
<td>No</td>
<td>No</td>
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<tr>
<td>ADSCs</td>
<td>Pig</td>
<td>Syn</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>BM Sca-1 Cells</td>
<td>Rat</td>
<td>Syn</td>
<td>No</td>
<td>No</td>
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<td>CMBs</td>
<td>Mouse</td>
<td>Xeno/Syn</td>
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<td>No</td>
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<tr>
<td>iPS-Derived CMs</td>
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<td>Xeno</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>BMCs</td>
<td>Rat</td>
<td>Syn</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>CDCs</td>
<td>Pig</td>
<td>Allo/Syn/Xeno</td>
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<td>TDCs</td>
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<tr>
<td>c-Kit+ Heart Cells</td>
<td>Pig</td>
<td>Syn</td>
<td>No</td>
<td>Yes</td>
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<td>CD34+ BMCs</td>
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</tr>
<tr>
<td>MPCs</td>
<td>Pig</td>
<td>Allo</td>
<td>Yes</td>
<td>Yes</td>
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</table>

Cell type, most advanced animal model tested, level of immune match, demonstration of cardioprotection in a clinically realistic model of acute myocardial infarction (yes/no) and clinical testing status (yes/no for any studies in patients, and yes/no specifically when tested clinically in acute phase of myocardial infarction [i.e., adjunctive to percutaneous intervention]). A PubMed search on “stem cells” and “acute myocardial infarction” yielded 446 preclinical citations (on March 24, 2017). These were sifted to identify studies in which cells were administered in vivo within 3 hours of coronary ligation if not reperfused, or within 2 hours of ischemia/reperfusion if reperfused. For any given cell type, only the earliest citation to have appeared in the search is cited. Variants of cell types subjected to various conditioning protocols or genetic alterations, or from multiple different species, are not parsed out individually. ADSCs indicate adipose-derived stem cells; AFSCs, amniotic fluid stem cells; BATDCs, brown adipose tissue–derived cells; BMNCs, bone marrow mononuclear cells; BM/Cs, bone marrow cells; BM Sca-1 cells, bone marrow Sca-1 cells; CD31+ BMDPCs, CD31+ bone marrow–derived progenitor cells; CD34+ BM/Cs, bone marrow CD34+ cells; CDCs, cardiosphere-derived cells; CMBs, cardiac mesangioblasts; EPCs, endothelial progenitor cells; ESC-derived...
CMs,\textsuperscript{54} ESC-derived cardiomyocytes; iPSC-derived CMs,\textsuperscript{58} iPSC-derived cardiomyocytes; MPAPCs,\textsuperscript{53} multipotent adult progenitor cells; MSCs,\textsuperscript{47} mesenchymal stem cells; TDSCs,\textsuperscript{61} tongue-derived stem cells; and UBMNCs,\textsuperscript{48} umbilical cord blood mononuclear cells.
Table 4
Summary of Allogeneic CDC Clinical Trials to Date

<table>
<thead>
<tr>
<th>Study Name</th>
<th>Study Design</th>
<th>No. of Subjects</th>
<th>Study Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALLSTAR</td>
<td>Phase 1 open-label allogeneic CDC in patients after myocardial infarction; single-vessel occlusive intracoronary delivery</td>
<td>14</td>
<td>Allogeneic CDCs safe, possibly effective in reducing scar size</td>
</tr>
<tr>
<td></td>
<td>Phase 2 multicenter randomized double-blind placebo-controlled trial of allogeneic CDCs in patients after myocardial infarction; single-vessel occlusive intracoronary delivery</td>
<td>142</td>
<td>Enrollment complete in follow-up</td>
</tr>
<tr>
<td>DYNAMIC</td>
<td>Patients with open-label heart failure with reduced ejection fraction, allogeneic CDCs; triple-vessel nonocclusive intracoronary delivery</td>
<td>14</td>
<td>Improved left ventricular ejection fraction and clinical status</td>
</tr>
<tr>
<td>HOPE-Duchenne*</td>
<td>Muscular dystrophy; randomized allogeneic CDCs vs controls; triple-vessel nonocclusive intracoronary delivery</td>
<td>25</td>
<td>Enrollment complete in follow-up</td>
</tr>
<tr>
<td>Regress-HFpEF†</td>
<td>Randomized double-blind placebo-controlled trial; allogeneic CDCs vs placebo; triple-vessel nonocclusive intracoronary delivery</td>
<td>40</td>
<td>Enrollment underway</td>
</tr>
<tr>
<td>ALPHA‡</td>
<td>Pulmonary hypertension; allogeneic CDCs vs placebo</td>
<td>26</td>
<td>Enrollment underway</td>
</tr>
</tbody>
</table>

ALLSTAR indicates the Allogeneic Heart Stem Cells to Achieve Myocardial Regeneration; ALPHA, Allogeneic CDCs for Pulmonary Hypertension Therapy; CDC, cardiosphere-derived cell; DYNAMIC, the dilated cardiomyopathy intervention with allogeneic myocardially-regenerative cells; HOPE-Duchenne, Halt Cardiomyopathy Progression in Duchenne; and Regress-HFpEF, Regression-Heart Failure with Preserved Ejection Fraction.


† E. Marban et al, unpublished data, 2017 (ClinicalTrials.gov. Unique identifier: NCT02941705).