The acute management of ST-segment-elevation myocardial infarction is now primarily focused on rapid restoration of myocardial perfusion to reduce myocardial infarct size, improve systolic function, and attenuate long-term postinfarction left ventricular (LV) remodeling. Indeed, in the contemporary reperfusion era, LV systolic function is now relatively preserved with postinfarction ejection fractions averaging 50%. As a result, postinfarction heart failure after primary coronary intervention now develops in fewer than ≈5% of patients and most have only New York Heart Association functional class I or II symptoms. In addition to early reperfusion, blockade of the neurohormonal axis with pharmacological therapies including angiotensin inhibition, β-blockers, aldosterone antagonists, and now nephrilysin inhibitors have significantly improved prognosis. These therapies further limit postinfarction remodeling and variably increase LV systolic function, resulting in improved functional capacity and increased survival. Yet, despite these significant therapeutic successes, a minority of patients go on to develop progressive LV dysfunction as well as recurrent myocardial infarction. This has motivated approaches to regenerate the myocytes and myocardium lost using cardiac cell therapies, which has become the focus of considerable preclinical and clinical investigation during the last 15 years. Although much research has focused on directly injecting cells into the myocardium, intra-arterially infused cardiopoietic stem cell therapies delivered at the time of myocardial reperfusion are emerging as promising candidates to improve LV dysfunction.²³

The feasibility of global intracoronary cell infusion without interrupting coronary flow was initially demonstrated over 10 years ago by Dawn et al. They showed that 1 million intra-arterially administered cKit⁺/lin⁻ cardiac stem cells (CSCs) delivered to the entire heart could attenuate LV remodeling in rats after ischemia and reperfusion. Not only was systolic function improved but EGFP labeled CSCs produced increased adult appearing myocytes in as little as 35 days in the infarct as well as remote regions of the heart. At a cellular level, there were measurable increases in global myocyte number with reductions in myocyte cell volume. A subsequent study using cKit⁺/CD45⁻ cardiac progenitor cells (CPCs) confirmed these relatively rapid beneficial physiological actions at a similar time point. There was dose dependency of functional effects after intra-arterially infused CPCs, which plateaued >0.75 million CPCs with higher doses of CPCs (≈6 million CSCs) causing increased mortality. Global intra-arterial CPC infusion elicited a prominent reduction in apoptosis within the infarct. Interestingly, reductions in apoptosis were equally as prominent in the normally perfused remote region of the heart. There were increases in myocyte proliferation leading to a net increase in BrdU+/α-SAR+ (α-SA⁺⁺) cells, reflecting small immature myocyte precursors. The long-term fate of the CPCs whether myocyte precursors developed into mature myocytes and the durability of the short-term functional improvement were unknown. Nevertheless, the administration of CSCs to patients using the stop-flow technique has demonstrated durable increases in myocardial function in patients with ischemic cardiomyopathy in the cardiac stem cells in patients with ischemic cardiomyopathy (SCIPIO) trial.

In this issue of the Journal, Tang et al⁷ provide important new insight into the long-term effects of intra-arterial CPC infusion at the time of reperfusion. Using echocardiography and state-of-the-art hemodynamic assessment, they show that the beneficial physiological effects previously demonstrated at 35 days after infarction were maintained at 3 months. Nevertheless, although favorable differences in function in CPC-treated versus control groups persisted during the subsequent 9 months, there was no further absolute increase in ejection fraction or fractional shortening demonstrated with CPC treatment. From an anatomic perspective, the preservation of function was accompanied by reductions in infarct size along with increases in viable LV mass within the risk region. The use of sex mismatched but otherwise syngeneic rats as CPC donors allowed the long-term CPC viability and differentiation into myocytes at 1 year to be clearly quantified. The results demonstrate persistence of CPC-derived cells for as long as 1 year and the absence of any tumorigenic effect (a critically important contribution for safety and clinical translation).
Surprisingly, although immunohistchemical staining demonstrated that the transplanted CPCs expressed contractile proteins, most α-SA<sup>POS</sup> cells continued to reflect small myocyte precursors with few attaining a size and morphometry consistent with a mature cardiomyocyte. The possibility that CPCs stimulated endogenous myocyte formation as the mechanism for functional improvement was explored using BrdC pulse studies administered for 1-month intervals at selected time points. Proliferating α-SA<sup>POS</sup> cells were increased throughout the heart after infarction but were ≈2-fold greater in CPC-treated hearts versus controls and much greater than BrdU<sup>+</sup> cells arising from sex-mismatched CPC donors. Nevertheless, like CPC-derived α-SA<sup>POS</sup> cells from donors, few endogenous BrdU<sup>+</sup> α-SA<sup>POS</sup> cells had a size and morphological appearance of a mature myocyte. Thus, although this study provides further evidence that CPCs demonstrate a durable effect on preventing the progression of postinfarction LV dysfunction, CPCs are not a source of new mature myocytes. Although the role of cKit<sup>+</sup> cells in cardiac regeneration continues to be controversial, this study adds support to an increasing number of preclinical studies assessing cell fate that have failed to identify significant numbers of de novo myocytes derived from cKit<sup>+</sup> cardiopoietic progenitor cells in other model systems.8,9 The small BrdU<sup>+</sup> α-SA<sup>POS</sup> cells that the authors interpret as immature myocytes do not result in mature myocytes, decline in frequency between 3 and 12 months and are not associated with functional improvement during this time period. Thus, rather than reflecting a delayed progression toward a mature myocyte phenotype, it seems that these immature myocytes may be in a futile cycle where they begin proliferating but then exit the cell cycle before ever reaching maturity. Alternatively, they may be undergoing rapid senescence.

If there are no mature BrdU<sup>+</sup> myocytes present after 1 year, where does the increase in myocardium demonstrated in the risk region of CPC-treated animals come from? The measurements of myocyte nuclear density and myocyte numbers demonstrate significant increases after CPCs but the size of the remaining myocytes (as well as the size cutoff used to exclude immature myocytes) was not provided. Lacking evidence of significant new mature myocyte formation from BrdU, there seems to be 2 possible mechanisms to explain increased myocardium in the risk region. The first is that most of the proliferation of CPCs into mature myocytes could have occurred in the first 2 months after therapy. Myocyte proliferation with increases in myocyte number and reductions in myocyte size have been demonstrated within 2 to 4 weeks after intra-arterial infusion after CSCs in rats<sup>4,5</sup> as well as cardiosphere-derived cells (CDCs) and mesenchymal stem cells in swine by others.<sup>10,11</sup> Because BrdC infusion in this study was not started until 3 months after CPC administration, the absence of mature de novo BrdU<sup>+</sup> cardiomyocytes would not be surprising. The second possibility is that the increased myocardium in the risk region actually reflects an antipapoptotic effect of CPCs administered shortly after reperfusion. Here, the authors’ recent study in the same model provides some insight.<sup>3</sup> Although the effect of CPCs on remodeling was dose dependent, a comparable dose range as used in this study (0.75–1.5 million CPCs) resulted in an increased infarct wall thickness, a reduction in infarct size and improved systolic function at 35 days. Although BrdU<sup>+</sup> α-SA<sup>POS</sup> cells were increased at this time, they were 100-fold lower than in this study (≈3/10,000 at 35 days versus ≈1000 in 10,000 nuclei at 3 months). At the same time, apoptosis at 35 days was increased and substantially reduced by CPCs (although the frequency of myocyte apoptosis was not reported). Thus, it seems likely that the increased myocardium in the risk region along with the preservation of function after CPCs is to some extent related to paracrine factors inhibiting apoptosis in the infarct as well as remote myocardium.

The complexity of studying myocardial repair in vivo rests on determining the relative importance of cell therapies in preventing death of existing myocytes versus promoting new myocyte formation. New myocytes can arise from the injected cells or by stimulating endogenous myocyte proliferation. It is straightforward to quantify the contribution of injected cells to new myocyte formation when sex-mismatched donors are a feasible approach as in the study of Tang et al.<sup>7</sup> In contrast, quantifying whether the new myocardium generated from endogenous cells reflects inhibiting cell death, myocyte cellular hypertrophy, or myocyte proliferation is challenging. The Table summarizes the complimentary approaches that can be used in this assessment. At the most fundamental histological level, morphometric approaches can quantify myocyte size, nuclear density, and the number of myocytes per gram of tissue. A reduction in myocyte size coupled with an increase in nuclear density (where the average number of nuclei per myocyte remains the same) indicates new myocyte formation because the number of myocytes per gram of tissue would have to increase. In contrast, a reduction in myocyte nuclear density along with an increase in myocyte size is compatible with myocyte cellular hypertrophy. Cellular hypertrophy can arise physiologically from normal growth in the young animals used in virtually all preclinical studies, myocyte loss from apoptosis or LV hypertrophy (regional or global). Because of this, attributing changes in viable LV mass to proliferation from cell therapy can be complex. For example, favorable myocyte regeneration could cause a resolution of postinfarction remodeling such that total viable LV mass and LV mass/body weight ratio is actually lower in treated versus untreated animals as in this study. Similar effects could also occur by inhibiting myocyte apoptosis. Assessing endogenous myocyte formation by quantifying BrdU<sup>+</sup> myocytes is attractive to identify de novo myocytes yet potentially problematic to interpret in vivo.<sup>12</sup> One concern relates to sensitivity of the technique because it is unknown how much BrdU is required to visualize

<table>
<thead>
<tr>
<th>Table. Morphometric and Anatomic Alterations Distinguishing Myocyte Regeneration, Ventricular Hypertrophy, and Physiological Growth</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Average myocyte size</strong></td>
</tr>
<tr>
<td>Myocyte number per gram or myocyte nuclear density</td>
</tr>
<tr>
<td>Left ventricular mass</td>
</tr>
</tbody>
</table>
a positive nucleus. The second issue is that continuous BrdU administration after treatment and appropriate costaining to identify myocytes is required to quantify the total number of myocytes formed. A final concern relates to the fact that cells can become positive because of DNA repair. In light of potential limitations of all approaches, the most definitive conclusions about myocyte cell proliferation can be made in a circumstance where apoptosis is absent and include measurements of regional and global LV mass, morphometric measurements (myocyte nuclear density, average myocyte size, and distribution) and administration of BrdU. If the latter is not possible, point estimates of proliferation using Ki67 staining and phospho-histone-H3 staining for mitosis can serve as surrogates with the caveat that they reflect instantaneous rates and likely change over time.

The study by Tang et al. adds to a growing body of preclinical literature demonstrating the efficacy of intra-arterially infused cardiopoietic cells in the absence of techniques, which require stop flow. Although this requires transient aortic occlusion and is technically challenging in the rodent heart, it is straightforward to accomplish in humans and large animals by direct intracoronary infusion. Suzuki et al. and Weil et al. demonstrated that continuous intracoronary infusion was efficacious without cessation of coronary flow and stimulated myocyte proliferation throughout the diseased but not normal heart after widespread intracoronary mesenchymal stem cell and CDC administration to swine with hibernating myocardium. Others have shown that transient occlusion or stop flow is not required for CSC or CDC uptake and demonstrated functional efficacy of CDCs administered without cessation of flow after acute myocardial infarction. Importantly, previous studies have identified substantial myocyte apoptosis, leading to myocyte loss in remote myocardium immediately after infarction in patients. The ability of widespread intracoronary cell therapies to prevent apoptosis and promote cell proliferation in ischemic as well as remote myocardium may provide a 2-pronged approach to prevent postinfarction remodeling and preserve LV systolic function. Global intracoronary stem cell infusion may also provide a relatively simple administration technique to treat patients with chronic ischemic as well as nonischemic cardiomyopathy. The latter possibility is being examined in an ongoing clinical trial using allogeneic intracoronary CDCs in humans.

Finally, although chronic cardiomyopathy can be treated electively using autologous cell platforms which are expanded ex vivo and then readministered to the donor, allogeneic cardiopoietic cell therapies are required to treat patients presenting with an acute myocardial infarction because cells need to be immediately available. Although allogeneic mesenchymal stem cells and allogeneic cardiosphere-derived cells have emerged as potential off-the-shelf products, it is unclear as to whether allogeneic CPCs will be a viable alternative to treat patients with an acute myocardial infarction like the syngeneic CPCs have been demonstrated to by Tang et al. Although this is worthy of future study, the long-term presence of CPCs and CPC-derived cells in the heart may actually be a disadvantage because these mismatched survivors would provide a stimulus for tissue immune rejection. Regardless of the specific cell therapy platform, it seems time to begin evaluating allogeneic cell therapy strategies that use global intracoronary cell infu- 

Sources of Funding

This study was supported by the National Heart Lung and Blood Institute (HL-055324, HL-061610, and F32HL-114355), the National Center for Advancing Translational Sciences (UL1TR001412), the Department of Veterans Affairs (H01BX002659), and the Albert and Elizabeth Rekate Fund in Cardiovascular Medicine.

Disclosures

None.

References

4. Dawn B, Stein AB, Urband K, et al. Cardiac stem cells delivered intra- 

vascularly traverse the vessel barrier, regenerate injured myocardium, and improve cardiac function. Proc Natl Acad Sci U S A. 2005;102:3766– 

3771. doi: 10.1073/pnas.0405957102.

CIRCHEARTFAILURE.115.002210.

S0140-6736(11)61590-0.
8. van Berlo JH, Molkentin JD. An emerging consensus on cardiac regenera- 


journal.pone.0113009.
12. Leone M, Magadum A, Engel FB. Cardiomyocyte proliferation in car- 

dian development and regeneration: a guide to methodologies and inter- 

doi: 10.1152/ajpheart.00559.2015.


**Key Words:** Editorials ■ heart failure ■ myocardial infarction ■ stem cells ■ ventricular remodeling
Widespread Intracoronary Cardiopoietic Cell Infusion: Treating at the Time of Myocardial Reperfusion to Prevent Rather Than Reverse Established Left Ventricular Dysfunction Moves Us Closer to Practical Clinical Translation

John M. Canty, Jr, Brian R. Weil and Gen Suzuki

Circ Res. 2016;118:1045-1048
doi: 10.1161/CIRCRESAHA.116.308518

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2016 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/118/7/1045

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation Research can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation Research is online at:
http://circres.ahajournals.org//subscriptions/