Function Follows Form
— A Review of Cardiac Cell Therapy —

Kenta Nakamura, MD; Charles E. Murry, MD, PhD

The investment of nearly 2 decades of clinical investigation into cardiac cell therapy has yet to change cardiovascular practice. Recent insights into the mechanism of cardiac regeneration help explain these results and provide important context in which we can develop next-generation therapies. Non-contractile cells such as bone marrow or adult heart derivatives neither engrant long-term nor induce new muscle formation. Correspondingly, these cells offer little functional benefit to infarct patients. In contrast, preclinical data indicate that transplantation of bona fide cardiomyocytes derived from pluripotent stem cells induces direct remuscularization. This new myocardium beats synchronously with the host heart and induces substantial contractile benefits in macaque monkeys, suggesting that regeneration of contractile myocardium is required to fully recover function. Through a review of the preclinical and clinical trials of cardiac cell therapy, distinguishing the primary mechanism of benefit as either contractile or non-contractile helps appreciate the barriers to cardiac repair and establishes a rational path to optimizing therapeutic benefit.

Key Words: Cardiomyopathy; Congestive heart failure; Ischemic heart disease; Stem cells; Transplantation

Despite significant advances in acute reperfusion and chronic pharmacotherapy, myocardial infarction (MI) remains a major cause of heart failure (HF) in Japan1 and the USA.2 The lost myocardium is replaced by fibrotic scar, leading to progressive left ventricular (LV) remodeling and further dysfunction. In contrast, there is a robust capacity for heart regeneration in lower vertebrates such as amphibians and fish,3 and neonatal mice (and possibly neonatal humans)4 also have significant capacity for cardiac regeneration and functional recovery.5–8 These insights suggest a novel opportunity for regenerative therapies to restore contractility, normalize function, and improve the quality and length of life. Although limited myocyte turnover in the post-natal mammalian heart9,10 has been reported to occur under specific conditions, such as chronic mechanical unloading of the left ventricle,11 this intrinsic regenerative capacity is limited to 0.5–1% of cardiomyocytes per year and is clearly inadequate to restore function following injury.12,13 Thus, healing of the adult heart is achieved through scar formation, ventricular remodeling and hypertrophy of the surviving cardiomyocytes, leaving the heart with a contractile deficit that too often progresses to HF.14

Although interventional and medical therapies have been responsible for significant reductions in mortality and slow the progression of ventricular dysfunction, they do not address the primary deficiency in HF, namely, the loss of contractile myocardium. Moreover, drug discovery for HF has slowed markedly, with only 2 new drugs [Entresto (sacubitril/valsartan) and Corlanor (ivabradine)] being approved in the past 2 decades. Medical therapy remains largely compensatory (e.g., diuresis, afterload reduction) or modestly disease modifying (e.g., neurohormonal regulation, suppression of vasoactive peptides or modulation of chronotropy), but none are curative. Consequently, ischemic heart disease and its complications are the number one cause of death worldwide.15

To address this unmet need, many groups around the world, our own included, have advocated for the development of therapies to promote cardiac regeneration. A multitude of cell-based, gene-based, cell-free and engineered tissue therapies have been proposed (Figure 1). Despite nearly 2 decades of clinical investigation, however, none of these has yet to transform contemporary cardiovascular practice. Here we offer a brief review of cardiac cell therapy, with an emphasis on clinical studies where available. We address mechanism of action and attempt to correlate this with clinical response. We conclude with suggestions for a path forward for cardiac stem cell therapy in the next decade. Engineered cell and tissue products, such as cellularized scaffold sheets, as well as attempts to directly reprogram injured myocardium (e.g., cardiac fibroblast transdifferentiation), are not discussed and have recently been recently reviewed elsewhere.16–19

Mechanisms of Action for Cardiac Cell Therapy

Cell therapy broadly aims to achieve 2 distinct but comple-
Circulate infarcted myocardium in preclinical models. The ability to remuscularize raises its own challenges, however, with numerous open questions that must be addressed for clinical viability. For example, the new myocardium may be arrhythmogenic as it heals in, and its ability to engraft long-term requires that either immune-compatibility or immunosuppression regimens be developed.

Non-Contractile Mechanisms of Benefit
The first cell type investigated for cardiac cell therapy was adult skeletal myoblasts, now over 2 decades ago. Early clinical trials showed promising benefit, but proved transient and appear to be mediated by non-contractile, paracrine activity. Pivotal clinical trials of autologous skeletal myoblast transplantation in patients with HF did not durably improve regional or global LV function and caused persistent ventricular arrhythmias, prompting abandonment of this cell type for therapy.

Figure 1. Function follows form: proposed cell types for therapeutic cardiac regeneration. Schematic showing various cell populations used in cardiac repair and their possible mechanisms of action. At present, only cardiomyocytes derived from pluripotent stem cells have the capacity to contribute direct contractile benefits to the injured heart. The existence of an endogenous cardiac progenitor cell is in question, given that purported populations such as c-kit+ and Sca1+ cells are conclusively not cardiopoietic, and unbiased genetic screens have not identified significant sources of new cardiomyocytes from stem cells.
Mechanistic Overview of Cardiac Cell Therapy

More recent efforts have shifted to other adult sources of cells purporting regenerative benefit through direct cell-to-cell and paracrine mechanisms, activating and stimulating endogenous regeneration and modulating repair processes. Numerous autologous and allogeneic adult cell types have been investigated clinically, including adult cells of cardiac origin such as cardiosphere-derived cells (CDCs) and cells of non-cardiac origin such as various BM-derived cells (e.g., BM-derived mononuclear cells (BM-MNCs), and mesenchymal stromal cells (BM-MSCs)). These so-called ‘first-generation’ cell types have been further refined as ‘second-generation’ cells composed of purified or stimulated subpopulations to potentiate their regenerative capacity. In all, some 15 types of adult cells have shown benefit in small animal models of MI. Development of these adult cell types have been accelerated to numerous phase 2 or 3 clinical trials within the past decade without clear mechanistic understanding (Table). With purported multiple effects in addition to regeneration, including direct cell-to-cell interaction and paracrine secretion of cardio-active cytokines and growth factors, investigators have expanded the indications for cell therapy from acute MI, where preclinical evidence was already lacking, to ischemic and non-ischemic cardiomyopathy, to refractory angina, peripheral artery disease and stroke. The beneficial effects of cell therapy do not appear restricted to adult stem cells and their derivatives, given that ectopic transplantation of engineered scaffolds containing pluripotent stem cell-derived cardiomyocytes have shown similar benefit in infarcted pigs, despite such grafts failing to integrate electromechanically with the host myocardium (grafts become vascularized but remain functionally uncoupled). Taken collectively, the poorly understood yet reproducible efficacy of non-contractile cell therapy in various disease states appears remarkably conserved across various cardiac and non-cardiac cells, suggesting that many or most cell types can exert a salutary paracrine effect when transplanted into the acutely infarcted heart. In one of the most rigorous
replicate many of the beneficial effects of cell therapy. The so-called secretomes consist of secreted factors, including growth factors, microRNAs (miRNAs) and extracellular vesicles/exosomes. Mechanistic studies of the secretome are just beginning, and whether these factors in isolation as cell-free products are as effective as cell-based therapy is mechanistic studies specifically of cell therapy, a preliminary report from Vagnozzi et al describes a novel innate immune response that explains the benefit of cell therapy through induction of a specific subset of macrophages to modulate healing in the infarct area.  

Media that has been conditioned by cultured cells can replicate many of the beneficial effects of cell therapy. The so-called secretomes consist of secreted factors, including growth factors, microRNAs (miRNAs) and extracellular vesicles/exosomes. Mechanistic studies of the secretome are just beginning, and whether these factors in isolation as cell-free products are as effective as cell-based therapy is possible.

### Table. Select Randomized Control Trials of Cardiac Regenerative Therapy

<table>
<thead>
<tr>
<th>Study</th>
<th>Design</th>
<th>No. of subjects</th>
<th>Cell type</th>
<th>Route</th>
</tr>
</thead>
</table>
| Wollert et al 2004 (BOOST)
Schachinger et al 2006 (REPAIR-AMI)
Janssens et al 2006 (Leuven-AMI)
Huikuri et al 2008 (FINCELL)
Tendera et al 2009 (REGENT)
Roncalli et al 2010 (BONAMI)
Hirsch et al 2011 (HEBE)
Traverse et al 2011 (LateTIME Trial)
Choudry et al 2016 (REGENERATE-AMI)
Süder et al 2016 (SWISS-AMI)
Quyyumi et al 2016 (PreSERVE-AMI)
Wollert et al 2017 (BOOST-2)
Traverse et al 2012 and 2018 (TIME)
Fernández-Avilés et al 2018 (CAREMI)
Mathur et al (BAMI, NCT01569178)
Ischemic cardiomyopathy
Menasche et al 2008 (MAGIC)
Perin et al 2011 (FOCUS-HF)
Perin et al 2012 (FOCUS-CCTR-I)
Hare et al 2012 (POSEIDON)
Assmus et al 2013 CELLWAVE
Heldman et al 2014 (TAC-HF)
Mathiasen et al 2015 (MSC-HF)
Patel et al 2016 (ixCell-DCM)
Bartunek et al 2017 (CHART-1)
Choudhury et al 2017 (REGENERATE-IHD)
Hare et al 2017 (POSEIDON-DCM)
CONCERT-HF (NCT02501811)
CHART-2 (NCT02317458)
DREAM HF-1 (NCT02032004)
CardiAMP (NCT02438300)
Assmus et al (REPEAT, NCT01693042) | SC, OL | 30 30 | Allo BM-MNC | IC |
| MC, DB | 95 92 | Auto BM-MNC | IC |
| SC, DB | 33 34 | Auto BM-MNC | IC |
| MC, DB | 39 38 | Auto BM-MNC | IC |
| SC, OL | 97 20 | Auto BM-MNC or CD34+CXCR4+ BM-MNC | IC |
| MC, OL | 52 49 | Auto BM-MNC | IC |
| SC, OL | 69 65 | BM-MNC/PB-MNC | IC |
| MC, DB | 58 29 | Auto BM-MNC | IC |
| MC, DB | 55 45 | Auto BM-MNC | IC |
| MC, DB | 95 55 | Auto BM-MNC | IC |
| MC, DB | 78 83 | Auto CD34+ cells | IC |
| SC, OL | 151 37 | Auto BM-MNC | IC |
| MC, OL | 79 41 | Auto BM-MNC | IC |
| MC, DB | 33 16 | Allo BM-c-kit+ CSC | IC |
| MC, DB | –175 –175 | Auto BM-MNC | IC |
| SC, OL | 67 30 | Skeletal myoblasts | TEP |
| SC, OL | 20 10 | Auto BM-MNC | TEN |
| MC, DB | 61 31 | Auto BM-MNC | TEN |
| SC | 31 0 | Allo or auto BM-MSC | TEN |
| SC, DB | 38 21 | Auto BM-MNC or auto BM-MSC | TEN |
| SC, DB, sham control | 40 20 | Auto BM-MSC | TEN |
| MC, DB, sham control | 58 51 | Proprietary auto BM-MSC and M2 macrophages | TEN |
| MC, DB, sham control | 120 151 | Auto BM-MSC (CpSC) | TEN |
| SC, DB | 70 35 | G-CSF/auto BM-MNC | IC or TEN |
| SC | 37 0 | Allo or auto BM-MSC | TEN |
| MC, DB, sham control | –200 –200 | Auto BM-MSC (CpSC) | TEN |
| MC, DB, sham control | –300 –300 | Auto BM-MSC (MPCs) | TEN |
| MC, DB, sham control | 167 83 | Potency-screened auto BM-MNC | TEN |
| MC, OL | –334 –334 | Auto BM-MNC | Repeated IC |

6MWT, 6-min walk test; AMI, acute myocardial infarction; allo, allogeneic; auto, autologous; BM, bone marrow; CpSCs, cardiopoietic stem cells; CSC, cardiac stem cells; CV, cardiovascular; DB, double-blind; HF, heart failure; IC, intracoronary; LVAD, left ventricular assist device; LVEF, left ventricular ejection fraction; MC, multicenter; MCS, mesenchymal stem cell; MLHFO, Minnesota Living with Heart Failure questionnaire; MNC, mononuclear cell; MPC, mesenchymal precursor cell; NA, not available; PB, peripheral blood; OL, open-label; QOL, quality of life; RCS, retrograde coronary sinus; SC, single-center; SDF-1, stem cell-derived factor 1; SERCA2a, sarcoplasmic reticulum Ca2+ ATPase; SPECT, single-photon emission computed tomography; TEN, transendocardial; VO2max, maximal oxygen consumption; VT/VF, ventricular tachycardia/fibrillation.

(Table continued the next page.)
Mechanistic Overview of Cardiac Cell Therapy

The cell therapy secretome that show promise in preclinical models of myocardial injury. miRNAs are highly conserved, single-stranded, short non-coding RNAs that regulate post-transcriptional gene expression by pairing with complementary sequences of messenger RNA. A catalog of miRNAs have been shown to mediate cardiomyocyte proliferation, apoptosis and repair following injury in vivo. Exosomes are small, extracellular vesicles <100 nm exocytosed by cells, including stem cells, and they contain various intracellular components of the donor cell, including bioactive lipids, proteins and RNA. Exosomes express specific cell surface markers and are effective vehicles for not yet clear. Early efforts focused on non-specific growth factors captured within extracellular matrices, and efforts to characterize specific effectors, such as the growth factor neuregulin 1 (NRG 1), vascular endothelial growth factor A (VEGF A), and fibroblast growth factor 2 (FGF2), have yet to show reproducible benefit. Failure of these early efforts at growth factor-mediated regeneration were thought to be caused by insufficient delivery and exposure of factors at target tissues, and more recent efforts have incorporated biomaterial delivery platforms or gene therapy methods.

<table>
<thead>
<tr>
<th>Cell no. (×10^6)</th>
<th>Timing (post-AMI)</th>
<th>Follow-up (months)</th>
<th>Primary outcome</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>2,460</td>
<td>5–7 days</td>
<td>6</td>
<td>Global LVEF</td>
<td>Positive</td>
</tr>
<tr>
<td>236±174</td>
<td>3–6 days</td>
<td>4</td>
<td>Global LVEF</td>
<td>Positive</td>
</tr>
<tr>
<td>172±72</td>
<td>24 h</td>
<td>4</td>
<td>Global LVEF</td>
<td>Negative</td>
</tr>
<tr>
<td>402±196</td>
<td>3 days</td>
<td>6</td>
<td>Global LVEF</td>
<td>Positive</td>
</tr>
<tr>
<td>178 (BM-MNC)</td>
<td>3–12 days</td>
<td>6</td>
<td>Global LVEF</td>
<td>Negative</td>
</tr>
<tr>
<td>98.3±8.7</td>
<td>9 days</td>
<td>3</td>
<td>Myocardial viability</td>
<td>Negative</td>
</tr>
<tr>
<td>296±164 (BM)</td>
<td>5–7 days</td>
<td>4</td>
<td>Global or regional LVEF</td>
<td>Negative</td>
</tr>
<tr>
<td>287±137 (PB)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>150</td>
<td>2–3 weeks</td>
<td>6</td>
<td>Global LVEF</td>
<td>Negative</td>
</tr>
<tr>
<td>60</td>
<td>&lt;24 h</td>
<td>12</td>
<td>Global LVEF</td>
<td>Negative</td>
</tr>
<tr>
<td>152</td>
<td>5–7 days or 3–4 weeks</td>
<td>12</td>
<td>Global LVEF</td>
<td>Negative</td>
</tr>
<tr>
<td>10±2</td>
<td>9 days</td>
<td>6</td>
<td>Resting myocardial perfusion</td>
<td>Negative</td>
</tr>
<tr>
<td>700–2,080</td>
<td>8.1±2.6 days</td>
<td>6</td>
<td>Global LVEF</td>
<td>Negative</td>
</tr>
<tr>
<td>147±17</td>
<td>3 or 7 days</td>
<td>6 and 24</td>
<td>Global or regional LVEF</td>
<td>Negative</td>
</tr>
<tr>
<td>35</td>
<td>5–7 days</td>
<td>1</td>
<td>Safety, all-cause mortality, reinfarction, HF hospitalization, VT/VF, stroke</td>
<td>Negative</td>
</tr>
<tr>
<td>NA</td>
<td>2–8 days</td>
<td>24</td>
<td>All-cause mortality</td>
<td>Recruiting, est. completion 2019</td>
</tr>
<tr>
<td>400 or 800</td>
<td>&gt;1 months</td>
<td>Global or regional LVEF</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>178</td>
<td>&gt;3 months</td>
<td>12</td>
<td>QOL, MLHFQ</td>
<td>Positive</td>
</tr>
<tr>
<td>100</td>
<td>&gt;1 month</td>
<td>6</td>
<td>LVESV, VO2 max, SPECT reversibility</td>
<td>Negative</td>
</tr>
<tr>
<td>20, 100, or 200</td>
<td>NA</td>
<td>1</td>
<td>Treatment-emergent serious adverse events</td>
<td>NA</td>
</tr>
<tr>
<td>205±110</td>
<td>&gt;3 months</td>
<td>4</td>
<td>Global LVEF</td>
<td>Positive</td>
</tr>
<tr>
<td>100 or 200</td>
<td>NA</td>
<td>12</td>
<td>Treatment-emergency serious adverse events</td>
<td>Neutral</td>
</tr>
<tr>
<td>77.5±67.9</td>
<td>&gt;6 weeks</td>
<td>6</td>
<td>LVESV</td>
<td>Positive</td>
</tr>
<tr>
<td>NA</td>
<td>&gt;3 months</td>
<td>12</td>
<td>Composite (all-cause death, cardiovascular hospitalizations, and worsening HF, etc.)</td>
<td>Positive</td>
</tr>
<tr>
<td>24</td>
<td>&gt;3 months</td>
<td>40</td>
<td>Composite (all-cause death, worsening HF, MLHFQ, 6MWT, LVESV, and LVEF)</td>
<td>Negative</td>
</tr>
<tr>
<td>115.1</td>
<td>&gt;3 months</td>
<td>12</td>
<td>Global LVEF</td>
<td>Positive for TEN</td>
</tr>
<tr>
<td>100</td>
<td>NA</td>
<td>12</td>
<td>Treatment-emergency serious adverse events</td>
<td>Neutral</td>
</tr>
<tr>
<td>NA</td>
<td>NA</td>
<td>12</td>
<td>Global LVEF, VO2 max, 6MWT, etc.</td>
<td>Paused</td>
</tr>
<tr>
<td>NA</td>
<td>NA</td>
<td>52</td>
<td>Composite (CV death, worsening HF, MLHFQ)</td>
<td>Paused</td>
</tr>
<tr>
<td>NA</td>
<td>NA</td>
<td>12</td>
<td>Time to HF exacerbation</td>
<td>Est. completion 2019</td>
</tr>
<tr>
<td>200</td>
<td>NA</td>
<td>12</td>
<td>6MWT</td>
<td>Recruiting, est. completion 2021</td>
</tr>
<tr>
<td>NA</td>
<td>NA</td>
<td>24</td>
<td>All-cause death</td>
<td>Recruiting, est. completion 2025</td>
</tr>
</tbody>
</table>
delivery of defined therapeutic effectors such as miRNA. Exosomes from murine cardiospheres are enriched in a specific miRNA and appears to be anti-apoptotic following MI, and studies of human cardiospheres appear to be equally promising. More recently, exosomes from human embryonic stem cell-derived cardiovascular progenitors (hESC-Pg) were shown to recapitulate the beneficial effects of their parent cell in a murine ischemic cardiomyopathy model. Once internalized, the hESC-Pg-derived exosomes promoted cell survival, cell proliferation, and angiogenesis in a dose-dependent manner. Theoretically, a cardiac-targeted exosome may also serve as an effective vehicle for delivery of defined therapeutic effectors such as miRNAs or small interfering RNAs.

Whether growth factors, miRNA and exosomes as cell-free preparations sufficiently recapitulate the paracrine benefits of cell therapy is unknown. Ultimately, characterization of a therapeutic secretome with a tailored delivery and retention platform may yield more potent benefits than the non-specific and transient effects of cell therapy observed to date.

**Contractile Mechanisms of Benefit**

Given their proven potential to remuscularize infarcted tissue, there is strong interest in pluripotent stem cells such as human embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs) as a renewable source of differentiated cardiomyocytes. Grounded in over 3 decades of basic science research, preclinical proof-of-concept studies of pluripotent stem cell-derived cardiomyocytes are increasingly promising as the field transitions from in vitro and small animal models to more relevant large animal studies. First isolated in 1998, human ESCs are isolated from the inner cell mass of the blastocyst in the early stages of embryogenesis. These cells retain the potential to differentiate into any somatic cell type, given the appropriate stimulation. Initially, there was hope that the heart milieu itself could provide either critical cell-cell cues or growth factors to guide ESCs to a cardiac phenotype and integrate into host myocardium. This notion was quickly dispelled, as injected ESCs into mouse myocardium formed teratomas rather than mature cardiomyocytes, in addition to eliciting immunogenicity and graft rejection.

Cardiomyocytes derived from ESCs, however, can be transplanted and survive in normal rodent hearts, and electrically couple with existing cardiomyocytes in a porcine model of atrioventricular conduction block. When transplanted into recipient rodent and guinea pig models after MI, there was a reproducible and durable improvement in LV function, and in the guinea pig, electrical coupling with the host myocardium. Efficient methods for high-purity, clinical-grade cardiomyocyte production from ESCs now allow extension of replacement cell strategies into preclinical large animal studies. Our group has transplanted 1 billion human ESC-derived human cardiomyocytes (hESC-CMs), approximating the cell loss during human MI, successfully in subacutely infarcted non-human primates. In that study, hESC-CMs were surgically injected into the hearts of immunosuppressed primates 2 weeks after infarction, resulting in significant remuscularization. The human graft became vascularized and electromechanically coupled with the host myocardium within 2 weeks post-transplant and remained durable up to 3 months.

More recent examples demonstrate the effectiveness and durability of human pluripotent stem cell (hPSC)-CM transplantation up 3 months. Shiba et al transplanted 400 million primate iPSC-derived cardiomyocytes into MHC-matched, immunosuppressed primates with follow-up to 3 months. Following transplantation, global contractility improved at 1 month and was sustained at 3 months compared with cell-free vehicle treatment. Importantly, this allogeneic transplantation study expands our understanding of the immunology of hPSC-CM grafts. With MHC matching, grafts were supported without rejection up to 3 months using an immunosuppression regimen commonly used clinically. The minimum immunosuppression required for MHC-matched, hPSC-CM allografts was not tested, but this study suggests that long-term engraftment is possible without the high levels of immunosuppression required for xenotransplantation.

Our group recently reported the long-term functional benefit of 750 million hESC-CM in non-human primates. An 8% improvement in ejection fraction (EF) was seen at 1 month, and at 3 months function had improved an additional 14%, essentially normalizing LV function (Figure 2). Control subjects negatively remodeled during the study with no improvement in LV function over time, as expected without background medical therapy. The persistent and cumulative benefit of engrafted hESC-CM, both subacutely and chronically, may reflect the importance of cellular engraftment to exert continuous benefit through contractile and non-contractile mechanisms. Dissecting the relative contribution of each in this setting is challenging. Whereas prior attempts at cardiac regeneration did not result in meaningful retention of cell product, and thus any benefit can be safely attributed to non-contractile benefit, hPSC-CM transplantation clearly results in durable engraftment. Although observation of large-scale remuscularization with contractile and electromechanically coupled grafts suggests a direct functional benefit, conclusive evidence requires careful genetic and pharmacologic studies to isolate contractile from non-contractile effects. Mechanistic studies to investigate the relative contribution of contractile and non-contractile effects will be important to refine this promising technology to the core mechanisms of benefit to maximize efficacy while minimizing complications such as malignant tachyarrhythmias.

A speculative model may be that the hPSC-CM transplantation uniquely matches the natural history of an evolving MI with both non-contractile and contractile effects. hPSC-CM may initially impart critical benefit to the subacute infarct via non-contractile, paracrine-mediated repair and moderation of injury. Indeed, small animal studies have failed to show benefit of remuscularization in chronic ischemic cardiomyopathy, suggesting a finite window of intervention for hPSC-CM remuscularization therapy to alter the long-term disease trajectory. As host cardiomyocytes are replaced with scar and the LV negatively remodels, the nascent cardiomyocyte graft is maturing and increasingly exerts contractile benefits, including force generation and structural support. This transition parallels the structural and electrical changes that occur over the 3-month period, yielding higher sarcomeric organization and electrical quiescence. Indeed, hPSC-CM cells are fetal-like at the time of delivery, which is a requisite phenotype to survive the hostile post-infarct myocardium and effectively engraft. The cells rapidly mature in vivo and ultimately contribute directly to function and positive remodeling.
Despite the promise of hPSC-CM transplantation, significant challenges to clinical translation remain, including scaling cell manufacturing to clinical levels, graft tolerance and immunosuppression, tumorigenicity, delivery, and most acutely, arrhythmogenesis. In earlier work with mice, rats and guinea pigs, no arrhythmias were observed after hESC-CM transplantation. When we moved into macaques, however, we observed a significant burden of ventricular arrhythmias. Electrophysiological studies indicate that the arrhythmias result from ectopic pacemaker activity by the graft cells, rather than reentry because of slow conduction. These arrhythmias typically last for 2–3 weeks, after which the heart returns to normal sinus rhythm. The lack of arrhythmias in smaller animals likely relates to host heart rate. Heart rates in model species range from 600 (mouse) to 400 (rat) to 250 (guinea pig) beats/min. Not until therapy was tested in non-human primate with a resting heart rate of 120–150 beats/min were ventricular arrhythmias reproducibly observed. Our current hypothesis is that arrhythmias stop when there is enough electrical maturation to drop pacemaking rates by the graft below that of the sinus node. Although the graft-induced ventricular arrhythmias are asymptomatic up to <10 kg non-human primates, they were not tolerated in a recent study of hESC transplantation in 20–30-kg pigs, with 2/7 pigs succumbing to an arrhythmic death. In that report, 1 billion hESC-CMs were surgically transplanted into pigs 3 weeks after MI, and all recipients experienced graft-induced ventricular tachyarrhythmias of worse severity than observed in non-human primates. As in previous studies, the graft-induced arrhythmias were transient and self-resolved within 1 month of transplantation. Electrophysiologic study was also consistent with increased automaticity rather than macro reentry as the etiology of the arrhythmia. Although not powered for efficacy, at 1 month post-transplantation there was no improvement in LV function in the cell-treated animals.

Other barriers to hESC-CM therapy include efficient and reproducible cell production and processing, graft survival without prohibitive immunosuppression and minimally invasive cell delivery, all of which must be addressed prior to clinical feasibility. To circumvent many of these issues, an alternative strategy of using a surgically placed epicardial patch seeded with ESC-derived cardiac progenitor cells is currently being tested in non-human primate with a resting heart rate. Heart rates in model species range from 600 (mouse) to 400 (rat) to 250 (guinea pig) beats/min. Not until therapy was tested in non-human primate with a resting heart rate of 120–150 beats/min were ventricular arrhythmias reproducibly observed. Our current hypothesis is that arrhythmias stop when there is enough electrical maturation to drop pacemaking rates by the graft below that of the sinus node. Although the graft-induced ventricular arrhythmias are asymptomatic up to <10 kg non-human primates, they were not tolerated in a recent study of hESC transplantation in 20–30-kg pigs, with 2/7 pigs succumbing to an arrhythmic death. In that report, 1 billion hESC-CMs were surgically transplanted into pigs 3 weeks after MI, and all recipients experienced graft-induced ventricular tachyarrhythmias of worse severity than observed in non-human primates. As in previous studies, the graft-induced arrhythmias were transient and self-resolved within 1 month of transplantation. Electrophysiologic study was also consistent with increased automaticity rather than macro reentry as the etiology of the arrhythmia. Although not powered for efficacy, at 1 month post-transplantation there was no improvement in LV function in the cell-treated animals.

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Clinical Trials of Cardiac Cell Therapy
The first clinical trials of cardiac cell therapy were performed nearly 20 years ago, with intramyocardial transplantation of skeletal myoblasts for ischemic cardiomyopathy. There have now been over 100 clinical trials of cell therapy for acute MI, over 90 for chronic ischemic cardiomyopathy, and 25 for non-ischemic cardiomyopathy. Before delving into specific cell types and disease targets, a few general comments are in order. Trials to date have generally used heterogeneous populations of adult cell types and have, for the most part, shown safety regardless of the specific investigational cell product, delivery approach, dosing protocol, or patient characteristics. Individual trials initially suggested efficacy, but those early trials were small without randomization, standardized enrollment criteria or endpoints. More recent trials with larger cohorts and superior study design have generally failed to convincingly show benefit over guideline-directed medical therapy. A recent Cochrane meta-analysis of 38 randomized control trials capturing 1,907 post-MI patients concluded that the current body of evidence for cell therapy is of low quality and lacking evidence for benefit by composite endpoint of mortality, non-fatal MI, and/or HF readmission. Long-term death >12 months and incidence of non-fatal MI were individually reduced with cell therapy, but confounded by relatively low event rates, small study cohorts, and non-standardized trial designs and adjudication. Numerous open questions remain for the clinical translation of cardiac cell therapy (Figure 3) and are discussed separately in this review. Representative trials of cell therapy are presented here to highlight the challenges facing further development of cardiac cell therapies (Table).

Bone Marrow-Derived Mononuclear Cells
BM-derived cells encompass a diverse cell population, of which BM-derived mononuclear cells (MNCs) and mesenchymal 'stromal' cells (MSCs) have been the most extensively studied in clinical trials. Regrettably, this line of investigation was established and perpetuated by the fraudulent work of Paolo Anversa and colleagues, who reported in 2001 that c-kit+ cells from BM regenerates injured myocardium following MI in mice. Despite being a single report in the mouse, this work was rapidly extended into human clinical trials (for a review of this unfortunate episode, see Chien et al). Despite compelling data contradicting the original findings by multiple independent groups in 2004, clinical trials continued as the purported biology switched from contractile to non-contractile mechanisms, with the alternative premise of direct cell-to-cell or paracrine mechanisms of action. These trials have generally yielded inconclusive or conflicting results, likely because of the small size of the phase 1/2 studies, heterogeneity of cell isolation, preparation, dosing, timing of therapy, baseline patient characteristics, endpoint adjudication, and study design. Routinely, modest and inconsistent-but-promising results in pilot and single-center phase 1 trials have not been reproduced in larger, multicenter phase 2 trials. For example, the original BOOST trial (Bone marrow transfer to enhance ST-elevation infarct regeneration, NCT00224536) of autologous intracoronary BM-MNCs randomized 60 STEMI patients to therapy or placebo and showed improvement in LV systolic function, but subsequently, no beneficial effect of BM-MNCs was found in the recent follow-up BOOST-2 trial (ISRCTN17457407) of 153 STEMI patients randomized to cell therapy or placebo.

To definitively answer the role of BM-MNCs as cardiac cell therapy, the BAMI (Bone marrow-derived mononuclear cells on all-cause mortality in Acute Myocardial Infarction, NCT01569178) trial was initiated by the group of Andreas Zeiher in Frankfurt, Germany. The culmination of over a decade of investigation in BM-MNCs for acute MI and LV dysfunction, BAMI is the first phase 3 trial intended to test benefit in all-cause mortality. Unfortunately, enrollment difficulties have reduced the initial target of 3,000 patients to 350. It is unclear whether the trial remains powered to be the pivotal outcome trial for adjunctive intracoronary infusion of autologous BM-MNCs in patients with acute MI and impaired LVEF in addition to revascularization and guideline-directed medical therapy. Recruitment for this multinational, open-label and random-
ized controlled trial commenced in 2013 and is anticipated to conclude in late 2019 after a 2-year minimum follow-up. For post-infarction ischemic cardiomyopathy, the REPEAT (REpetitive Progenitor cCell therapy in Advanced chronic heart failure, NCT01693042), is a multicenter, open-label, randomized controlled trial of 676 patients comparing single vs. repeat intracoronary infusion of BM-MNCs powered for all-cause mortality, with completion anticipated in 2025 after a 2-year minimum follow-up.196 If the BAMI and REPEAT trials are successfully completed, their outcomes will hopefully definitively answer the efficacy of BM-MNCs in acute MI and ischemic cardiomyopathy, respectively.

**Bone Marrow-Derived Mesenchymal Stromal Cells**

Another BM-derived population, mesenchymal stromal/stem cells and their derivatives, have been evaluated extensively in clinical trials. These fibroblastic cells support hematopoiesis through cytokine secretion and can readily be expanded from BM aspirates or biopsies. We prefer the universally-adopted nomenclature, MSCs, to avoid conflating their biology with true stemness, given their heterogeneity and limited ability to self-renew and exhibit multipotency beyond specific niches.107,108 The PROMETHEUS (Prospective Randomized Study of Mesenchymal Stem Cell Therapy in Patients Undergoing Cardiac Surgery, NCT00587990) trial109 studied 6 patients undergoing cardiac surgical revascularization with low (n=2) or high (n=4) dose BM-MSC therapy delivered by transepicardial injection into akinetic, unrevascularized territories. The MSC injections were well tolerated and regional cardiac function improved compared with revascularized territories by 18 months following the procedure. A significant caveat is that all patients also underwent coronary artery bypass grafting, a procedure that is known to improve cardiac function in chronically ischemic hearts, and global cardiac function universally improved as expected. It is thus not possible to conclusively distinguish the effects of MSC treatment from surgical revascularization in this study. Several trials have evaluated transendocardial (TEN) injection of MSCs in ischemic and dilated cardiomyopathy. The TAC-HFT (Transendocardial Autologous Mesenchymal Stem Cells and Mononuclear Bone Marrow Cells in Ischemic Heart Failure Trial, NCT00768066) trial compared autologous MSCs with BM-MNCs and placebo,110 and the POSEIDON (Phase 1/2, Randomized Pilot Study of the Comparative Safety and Efficacy of Transendocardial Injection of Autologous Mesenchymal Stem Cells Versus Allogeneic Mesenchymal Stem Cells in Patients With Chronic Ischemic Left Ventricular Dysfunction Secondary to Myocardial Infarction, NCT01087996) trial compared autologous and allogeneic MSCs in ischemic cardiomyopathy.111 As phase 1/2 studies, TAC-HFT and POSEIDON were powered primarily for and demonstrated safety. Efficacy signals were mixed and warrant an adequately powered phase 2/3 study. The POSEIDON-DCM (Percutaneous Stem Cell Injection Delivery Effects on Neomyogenesis in Dilated Cardiomyopathy, NCT01392625) trial compared autologous and allogeneic MSCs in dilated cardiomyopathy with similar safety outcome.112 A recent meta-analysis of these single-center trials supported the individual trial conclusions of safety, with suggestion of functional benefit in dilated cardiomyopathy and improved LV remodeling in ischemic cardiomyopathy.113 Evidence for efficacy requires adequately powered phase 2/3 trials.

Notably, the multicenter, double-blind, sham-controlled trial ixCELL-DCM studying a proprietary therapeutic cell product consisting of autologous BM-MSCs and a subset of macrophages (M2) in 126 patients with diluted ischemic cardiomyopathy demonstrated a positive effect on the primary composite endpoint of all-cause death, cardiovascular hospitalizations, and HF decompensation at 1 year, though driven primarily by increased HF events in the sham control cohort.114 Growth factor/cytokine stimulation of BM-MSCs was hypothesized to enhance the ability of MSCs to repair the heart, and this notion led to the C-CURE (Cardiopoietic Stem Cell Therapy in Heart Failure, NCT00810238) trial. The study used cytokine-treated MSCs for patients with ischemic cardiomyopathy, demonstrating safety and suggestion of efficacy, including significantly improved LVEF, reduced LV end-systolic volume (LVESV), and improved 6-min walk test (6MW1) compared with standard of care.115 The follow-up study, the European-based CHART-1 (Congestive Heart Failure Cardiopoietic Regenerative Therapy, NCT01768702) trial, is a double-blind, sham-controlled study of 315 patients with ischemic cardiomyopathy treated with transendocardial injection of cytokine-treated MSCs.116 Although safety was again demonstrated, the trial failed to achieve the prespecified primary composite endpoint (all-cause death, worsening HF, LVEF, LV end-diastolic volume, 6MW1), compared with sham control. The negative result of CHART-1, the largest trial of its kind to date, has raised several questions, including the choice of cell type (autologous source with variable regenerative potency), untested dosing and delivery, and insufficient power. The North American counterpart, CHART-2 (NCT02174358), sought to evaluate whether transendocardial injection of cytokine-treated MSCs is effective in a more severe subgroup of patients with greater LV dilatation but appears to be on hold. Finally, the multicenter phase 2 trial DREAM HF-1 (Efficacy and Safety of Allogeneic Mesenchymal Precursor Cells [Rxdestromectol-L] for the Treatment of Heart Failure, NCT02032004) is recruiting to randomize 600 patients with ischemic cardiomyopathy to treatment with mesenchymal precursor cells, a proprietary subset of MSCs, or placebo with a study completion date of late 2019.

**Cardiosphere-Derived Cells**

CDCs are a heterogeneous population of fibroblastic cells derived from heart explants, which have shown benefit in preclinical testing and are in early clinical tests. The term cardiosphere refers to a stage of cell expansion where the cells are grown as 3D spheroids. In preclinical studies ranging from mice to pigs, CDCs improved LV function following infarction.116-118 The single-center CADUCEUS (Cardiosphere-Derived Autologous StemCells to Reverse Ventricular Dysfunction, NCT00893360) trial119,120 randomized patients post-MI with impaired LVEF <40% to cell therapy or placebo. At 12 months, therapy failed to improve hard and soft functional endpoints compared with placebo, although there was MRI evidence for significant shrinkage of the infarct scar size. The follow-up multicenter phase 2 trial ALLSTAR (Allogeneic Heart Stem Cells to Achieve Myocardial Generation, NCT01458405)121 was recently terminated early for futility to achieve the primary endpoint of scar size by MRI after interim analysis at 6 months. As a result, CDC therapy is no longer being developed for ischemic cardiomyopathy and is instead being investigated...
for dilated cardiomyopathy in end-stage Duchenne muscular dystrophy as part of the HOPE-2 trial (NCT03406780) following safety and signals of efficacy for cardiac and upper limb function in the initial phase 1/2 Halt Cardiomyopathy Progression (HOPE)-Duchenne trial. The trial was recently resumed after a voluntary hold following a severe adverse event of anaphylaxis.

**Cardiac c-kit+ Cells**

A large body of preclinical literature touts the existence of endogenous cardiac stem/progenitor cells that mediate cardiomyocyte renewal and induce widespread regeneration after transplantation. Unfortunately, this work was again championed by Piero Aversa and colleagues, who committed widespread fraud and have had more than 30 papers retracted. The recently retracted SCIPIO (Stem Cell Infusion in Patients with Ischemic cardiomyopathy, NCT00474461) trial subsequently was led by Anversa, Roberto Bolli and colleagues, and was the first randomized study of intracoronary autologous c-kit+ cells, derived from atrial biopsies, in ischemic cardiomyopathy. The study of 20 treatment and 13 control patients with LVEF <40% were randomized to cell therapy or placebo at 4 months post-MI at the time of surgical revascularization. The authors reported significant improvement in LVEF with treatment compared with placebo at 1 and 2 years. Following questions of data integrity by the article’s publisher, the *Lancet*, the study was flagged with a Letter of Concern in 2014 and formally retracted in 2019.

Combination therapy of intramyocardial injection of BM-MSC and c-kit+ cells is being studied in the multicenter phase 2 trial CONCERT-HF (Combination of Mesenchymal and C-Kit+ Cardiac Stem Cells as Regenerative Therapy for Heart Failure, NCT02501811). This trial was originally slated to enroll 144 patients with ischemic cardiomyopathy randomized to cell therapy or placebo at 4 months post-MI at the time of surgical revascularization. In a cautionary lesson for a field widely believed to be safe, 1 patient died from pericardial tamponade after cardiac biopsy to harvest the c-kit+ cells perforated the right ventricle. Enrollment for this NIH-sponsored study was halted when the Aversa scandal came to light and the premise of the study was invalidated. At the time of writing, the data safety monitoring board has decided to pause further enrollment but will complete the cardiac injections in patients from whom c-kit+ cells have already been isolated.

**Open Questions in Optimizing Cardiac Cell Therapy**

The generally modest and often negative results of adult cardiac cell therapy trials have been attributed to a number of reasons, both specific to the therapy itself and the study design used. However, although each of these interacting variables could have significant influence on the efficacy of cell therapy, it is not clear if, or how these vexing issues will ever be resolved, given the accumulating neutral and negative trials for adult cell therapy. In our opinion, the weight of the data supports a shift to strategies with better established mechanisms and preclinical validation.

**Cell Type and Source**

Multiple competing cell types have been proposed for cardiac cell therapy, without comparative study to assess superiority or preclinical studies carefully defining the properties responsible for benefit. What is clear is that studies comparing various cell therapies must respect the presumed mechanism of benefit in designing such studies. For example, comparisons of contractile (i.e., giving new cardiomyocytes) and non-contractile (all other cell types) therapies cannot be conducted at similar dose ranges. This is because true remuscularization requires delivery and survival of hundreds of millions to billions of cardiomyocytes, whereas non-contractile cells, which survive only transiently, require far fewer cells. Combination therapy may also provide additive benefits, given the diverse activity of the various cell types, but at present there are not sufficient preclinical data to identify the most effective combinations.

Another important consideration is autologous vs. allogeneic source. Although autologous sourcing avoids the immunocompatibility issues of allogeneically sourced cells, patient comorbidities such as advanced age, diabetes, smoking, and obesity may limit the potency of adult cells. The ongoing CardiAMP trial (NCT02438306) attempts to address the issue of potency by screening harvested cells prior to use. Autologous therapies may be feasible for subacute or chronic indications requiring limited cell expansion, but this approach is currently impractical for replacement strategies requiring billions of cells or for diseases that require treatment within weeks of presentation such as recent MI. In contrast, allogeneic cells allow for “off-the-shelf” availability for acute and subacute therapy, as well as product development under strict and consistent quality-controlled conditions and ease of scalability. Chronic immunosuppression is currently required for durable survival of allogeneic cells, although the degree of immunosuppression required for cardiomyocyte transplantation is poorly understood because the bulk of the preclinical data is in xenotransplantation (e.g., human cells transplanted into non-human primate heart). Efforts to engineer allogenic cells for reduced immunogenicity were recently reported in humanized mice. Such advances may allow allograft survival with easily tolerable immunosuppression regimens like that prescribed routinely for chronic autoimmune disorders such as rheumatoid arthritis.

**Cell Dose**

For replacement therapies such as proposed with hPSC-CM, dose escalation until lost myocardium is fully remuscularized seems logical. Accordingly, preclinical studies in macaque monkeys have administered 400 million to 1 billion ESC-derived cardiac myocytes, although full dosing studies in appropriate human-sized models have not occurred. For adult stem cell products with primarily non-contractile effects, the appropriate dosing is unclear. A reverse dose response has been reported in the POSEIDON trial, likely because of vascular obstruction and maladaptive angiogenesis with intracoronary administration, whereas higher doses appear beneficial for transcendocardial delivery, as observed in the TRIDENT trial. Variable cell potency further complicates the issue of cell dosing, particularly for autologously sourced cells. In the absence of well-powered and appropriately controlled dose-response trials, optimal dosing remains unknown and needs to be empirically derived for each study.

**Timing of Therapy**

Although often reported interchangeably, administration
of cells in the acute, subacute or chronic phases following MI would be expected to work by significantly different mechanisms and require different properties. Acute administration aims to modulate inflammation, vascularization and host cardiomyocyte apoptosis, whereas subacute or chronic therapy aims to augment myocardial repair and replace lost myocardium. Although not studied systematically, a meta-analysis of adult cell therapy suggests that 2–8 days post-MI is the most favorable window to modulate injury response and potentiate repair mechanisms. Interestingly, preclinical studies have generally focused on the subacute phase, occurring at 2 weeks after the resolution of acute phase inflammation and reperfusion injury that may be hinder engraftment. In fact, iPSC-CM transplantation in rats and guinea pigs has only been beneficial in this subacute phase, as studies in the chronically infarcted heart have been negative despite persistent cell engraftment.98–99

Route of Administration
Several routes of cell administration have been studied preclinically, including intravenous, intracoronary, percutaneous transendocardial, transcoronary endocardial, retrograde intracoronary sinus with antegrade coronary obstruction, and open surgical epicardial. By far the most common route of adult cell delivery post-MI is intracoronary infusion, given the central role for catheter-based revascularization in contemporary practice. However, intracoronary cell delivery is associated with rapid washout and little retention of cells, which may be sufficient for paracrine effects but not remuscularization. Large doses of stem cell-derived cardiomyocytes cannot be delivered by intracoronary infusion, because they would obstruct microvascular beds and cause significant ischemia. Intramyocardial injection, whether epicardial or endocardially delivered, is superior for cell engraftment,98 but poses a higher risk of arrhythmia and perforation of the infarcted myocardium after MI and thus may be more appropriate for subacute and chronic therapy where remuscularization is the goal. More granular issues of optimal target tissue, such as peri- vs. intra-infarct myocardium or transmural infarcted vs. stunned/hibernating myocardium, are also largely unexplored.

Patient Characteristics
The patient characteristics affecting myocardial substrate have not been studied systematically, yet undoubtedly they must affect response to cell therapy, particularly for autologous cell products where poor patient parameters impair both cell potency and host receptivity. Patient comorbidities such as advanced age, diabetes and injury-related features such as ischemia time, reperfusion, revascularization strategy, and residual ischemic burden are highly variable between patients, influencing the natural history of injury and subsequent HF as well as the hostility of the milieu into which cells are transplanted. Given the small size of existing studies and heterogeneity with respect to inclusion criteria it is unlikely this will be resolved soon.

Conclusions
The disappointing clinical experience with non-contractile cardiac cell therapy reveals a fundamental lack of mechanistic insight and provides a cautionary lesson for investigators considering first-in-human trials of remuscularization therapy. Although initially thought to directly contribute to force generation, the purported mechanism of adult cell therapy shifted to indirect cell-to-cell and paracrine signaling with increasing futility to refine subpopulations that are more potent and cardiopoietic. Attempts to remuscularize the failing heart with adult cells from the BM or heart proved elusive because of myriad challenges, the foremost of which is the fact that adult cells do not differentiate into cardiomyocytes. The results of the subsequent clinical trials are thus better understood through the lens of expected biology and mechanistic basis. The most promising preclinical investigations of cardiac remuscularization therapy returns to the premise that meaningful and durable recovery of injured myocardium requires genuine and direct regeneration of lost myocardium to restore contractility.89,128,132

The extensive experience with adult cell therapy, however, provides a reassuring framework of infrastructure for the safe delivery of cells in such trials, as well as a cautionary lesson in better understanding of underlying biology and establishing protocols through careful preclinical investigation and validation. The non-contractile benefit observed with adult cells warrants further basic research to identify the mechanism of action. Once better understood, these pathways may be an important adjunctive benefit for therapies based on contractile cell replacement. The recognized challenges related to arrhythmogenesis, immunosuppression and efficient cell production with direct cardiac cell replacement require solutions before clinical viability, but are intrinsic to the fundamental strategy of true cardiac remuscularization. Within this established mechanism, the field can advance to address these known and surmountable barriers with direction and purpose.

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