Myocardial aging is a constellation of concurrent processes occurring at organ, cellular, and molecular levels. Despite this complexity, differences become readily identifiable by comparing phenotypic characteristics of mammalian cardiomyocytes and cardiac stem cells in young versus old hearts. One hallmark unique to the early postnatal myocardium is increased cellular proliferation, resulting in formation of new myocytes. Although research is ongoing to identify when proliferation subsides in the postnatal myocardium, current findings indicate that the murine heart experiences a proliferative burst around 2 weeks, whereas the human heart experiences proliferation in smaller growth spurts throughout adolescence. It is hypothesized that the regenerative capacity of the neonatal mammalian heart is dependent on the sympathetic nerve, and poorly functioning nerve structure in adult myocardium impairs the heart’s capability to regenerate. Later in life, de novo myocyte formation in mammals is severely limited by the suboptimal milieu of struggling myocardium and accumulation of poorly functioning senescent myocytes. Senescence also exacts a toll on the cardiac stem

Empowering Adult Stem Cells for Myocardial Regeneration V2.0
Success in Small Steps
Kathleen M. Broughton, Mark A. Sussman

Abstract: Much has changed since our survey of the landscape for myocardial regeneration powered by adult stem cells 4 years ago. The intervening years since that first review has witnessed an explosive expansion of studies that advance both understanding and implementation of adult stem cells in promoting myocardial repair. Painstaking research from innumerable laboratories throughout the world is prying open doors that may lead to restoration of myocardial structure and function in the wake of pathological injury. This global effort has produced deeper mechanistic comprehension coupled with an evolving appreciation for the complexity of myocardial regeneration in the adult context. Undaunted by both known and (as yet) unknown challenges, pursuit of myocardial regenerative medicine mediated by adult stem cell therapy has gathered momentum fueled by tantalizing clues and visionary goals. This concise review takes a somewhat different perspective than our initial treatise, taking stock of the business sector that has become an integral part of the field while concurrently updating state of affairs in cutting edge research. Looking retrospectively at advancement over the years as all reviews eventually must, the fundamental lesson to be learned is best explained by Jonatan Mårtensson: “Success will never be a big step in the future. Success is a small step taken just now.”

Key Words: adult stem cells cardiovascular disease clinical trial therapy myocardium

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cell (CSC) population that precipitates a loss of regenerative capacity. These multifaceted issues developing from age and injury lead to reduced contractility and cardiac output, culminating in cardiovascular disease (CVD).

Simultaneously, CVD can develop not only with age, but also from chronic disease (diabetes mellitus, obesity, hypertension), habitual stressors (smoking, poor diet, alcoholism), or genetic predisposition. Consequently, CVD is the leading cause of morbidity and mortality worldwide with ≈400,000 new cases per year (a total diagnosis affecting over 28 million people). The total US healthcare cost for diagnosis and treatment of CVD exceeds $32 billion per year with a 50% mortality rate within 5 years of diagnosis. Of individuals having CVD, ≈5 million people have heart failure (HF). Deaths from HF exceed those from either breast or colon cancer. The most common cause of HF in the Western World is ischemic heart disease fundamentally rooted in loss of functional cardiac tissue. Therefore, regeneration of cardiac tissue to alleviate the underlying cause of CVD and HF is a major public health concern.

Despite medical interventional therapy (statins, β-adrenergic blockers, angiotensin-converting enzyme inhibitors, aspirin, clopidogrel, aldosterone antagonists, etc), the prognosis of patients with ischemic HF remains poor. Consequently, new approaches are needed to reduce mortality and morbidity of patients to account for considerable enthusiasm and interest in cell-based therapy. If cell therapy is beneficial in patients with ischemic cardiomyopathy, such benefits are likely synergistic and complementary to those of standard pharmacological therapy. Cell therapy mechanisms include, for example, formation of new myocytes, secretion of paracrine factors, reduction of fibrosis, enhancement of contractility, and promotion of endogenous regeneration. These mechanisms do not involve neurohormonal pathways targeted by standard pharmacological therapy (blockade of β-adrenergic receptors, aldosterone antagonism, ACE inhibition, etc). Differences in underlying mechanisms of action imply that cell therapy likely imparts benefits additive to those of standard medical therapy. Indeed, according to the latest and most authoritative meta-analysis, the trials of cell therapy in patients with ischemic cardiomyopathy produced beneficial effects in response to traditional medical therapy. However, salutary effects produced by stem cell therapies have been modest and variable. With a goal of full functional restoration in patients treated for HF, the pivotal question centers on which direction(s) is(are) necessary to advance cellular therapy toward achieving greater returns on investment.

First-generation adult stem cell therapies focused on single cell types, including bone marrow stem cells, mesenchymal stem cells (MSCs), CSCs, or similar. Paradoxically, clinical trial results were most promising in terms of improved cardiac function and viable cardiac tissue with the use of CSCs, although cardiac progenitor cells (CPCs) have not advanced as rapidly as other cell types in the clinical setting. Currently, clinical trials are gearing up for combinatory stem cell therapies, such as MSCs and CSCs. Research in the use of MSCs and CSCs have also led to a new branch of potential therapy with secretome. Ongoing basic science and preclinical trials will continue to focus on enhanced stem cells, combinatory stem cells, and secretome, which will lead to the next generation of clinical trials. Continued persistence, transparency in reporting, and avoidance of hyperbolic promises will be essential to drive CSC research toward interventional therapies that provide long-term benefit.

This review is intended as an overview of progress of adult stem cell therapies toward treatment of HF. Use of induced pluripotent stem cells or embryonic stem cells as a means to treat HF will not be discussed because of the fundamental biological differences that exist between these and adult stem cells, as well as markedly distinct challenges in clinical implementation. The assessments presented here will focus upon use of adult stem cells to treat heart disease, with particular attention paid to aspects of private enterprise and governmental regulation. Next, factors that influence clinical results for use of stem cells as a therapeutic treatment will be covered, culminating in an examination of future prospects for adult stem cells in treatment of HF.

### Strategic Management

#### The Marketplace

As is typical for technology and innovation, a degree of risk exists during testing and trial phases. For biotechnology, a great measure of protection and respect for life is established to minimize such risks during research development and clinical testing through internal and external regulatory bodies. Invasive stem cell therapies are regulated by the United States Food and Drug Administration (FDA) as a drug and, therefore, face very stringent preclinical studies to demonstrate safety and efficacy before approval for a Phase I Clinical Trial. The United States has progressed at a steady pace toward development of stem cell therapies for treatment of HF. Progress of cellular therapies to treat heart disease continues to increase in clinical trials, dependent on the cellular therapy applied. With the overall population of heart disease continuing to grow and the average age of a person diagnosed with heart disease decreasing, responding successfully to this growing market becomes increasingly urgent.

The global market for adult stem cells is led by the US market, with an estimated $21.6 billion of $32.0 billion market share in 2013 and a projected $57.34 billion of $94.13 billion by 2018. The market is composed of broad
categories, including the technology (adult stem cell acquisition, production), products within the technology (cells, exosomes, mRNA, etc), the application of the products (regenerative medicine, drug discovery and development, research, etc), and the geographic regions (North America, South America, Asia-Pacific, etc), involved with the development and implementation of the application. Within the field of stem cell therapies, adult stem cells constitute 80% of the total stem cell market. The appeal of adult stem cells is in the fact that they are multipotent and have plasticity, and different tissue types can arise from differentiation of these cells. This is in contrast to embryonic stem cells or induced pluripotent stem cells, which are pluripotent, can give rise to a greater number of tissue types that are not easily controlled during differentiation, and can potentially lead to abnormal growth and tumor formation. Advantages in the fundamental characteristics of adult compared with embryonic stem cells have led to rapid implementation of clinical trials with the former, whereas the latter remain in preclinical development.

With a large market for stem cell technology, the funding to support the research is drawn from several sources in the private sector (direct corporate research funds, contracted research from corporations at institutes, universities or start-up companies, and investors, through angel funding, venture capitalists, investment bankers) and the public sector (federal, state, and nonprofit funding). MoneyTree, a means to review venture capital investment activity in the United States, reports the Biotechnology sector holds ≈13% of the investment marketplace, with a Q3 2015 investment of $2.13 billion and Q4 2015 investment of $1.46 billion. Historical data reported by MoneyTree displays an upward long-term growth trend in venture capital investment in the biotechnology sector, but investment with respect to stem cell research or adult stem cell therapies in the treatment of HF is not reported. The private sector has consistently funded more medical and health research in the United States, with a total R&D investment in 2012 for the combined pharmaceutical, biotechnology, and medical technology industries at $69.17 billion, whereas the combination of federal government entities invested $41.02 billion in 2012 (National Institutes of Health [NIH] provides the greatest investment of $30.01 billion).

Federal funding provided by the NIH for overall stem cell research can be viewed in the Research Portfolio Online Reporting Tools (RePORT) system. Data can be obtained regarding total funding invested in heart disease or CVD as well as regenerative medicine or stem cell research as a general search term, but specifics for NIH funding allocated to adult stem cell research for cardiovascular regeneration is not readily procured without data mining. Basic science at NIH is funded by classical research, training, and fellowship grants, and program project grants and the R43/R44 grant programs are specifically geared for start-up funding of early-stage small businesses. Small business programs are available at other federal agencies, including Department of Defense and Department of Energy but the NIH governs small business funding for biological or medical research involving the use of animals or human populations. State-level initiatives for funding adult stem cell research vary by state. California is heavily invested in stem cell research with approval of California Proposition 71 in 2004 to spend $3 billion in 10 years, financed by state general obligation bonds and managed by the California Institute for Regenerative Medicine. California Institute for Regenerative Medicine has invested over $1.95 billion in adult stem cell research and continues to operate for both education and research-related initiatives. In New York, the New York Stem Cell Foundation, founded in 2005 for basic and translational scientific research related to stem cell biology, is a 501(c)(3) nonprofit organization and funded through private philanthropy. The state publicly allocates funds through the Empire State Stem Cell Trust Fund to the New York State Stem Cell Science Program with an initial 2007–08 appropriation of $100 million with an additional $50 million per year for 10 years. Additional states actively funding stem cell research include, and not limited to, Connecticut, Ohio, Maryland, Massachusetts, Illinois, Minnesota, Wisconsin, Rhode Island, and New Jersey, where many are members of the Interstate Alliance on Stem Cell Research. Funding for early-stage research business has been achieved through private or public funding but a blend of both mechanisms lends to the overall success of the business as the business transitions from fundamental R&D toward product commercialization.

Development of adult stem cells as a therapeutic product to treat HF has led to several biotechnology start-up companies. Comparison of companies with adult stem cell therapies focused on increasing vascularization of heart tissue or building of new cardiac tissue to replace damaged cardiac tissue can be organized by various characteristics, such as the current clinical trial phase or the origin of the cell (see Figure 1).
and Table). The table includes a column titled Clinical Trial to reference recent trial(s) associated with the company based on information provided in ClinicalTrials.gov and the company website. Figure 1, in turn, reflects the company’s advancement to commercialization of its product based on the listed trial. For these and future companies to be successful in achieving FDA approval with a cellular therapy to treat HF, a clear understanding of cellular mechanisms that influence incorporation into heart tissue is necessary. When we, as a research community, better understand stem cells, we will be able to optimize clinical outcomes for patients using an approach of personalized medicine.

The Product

Stem cell therapy for cardiac repair holds great promise, but the ability of stem cells to repair damaged myocardium declines with age and is characterized by impaired functional reserve of the endogenous stem cell pool because of exhaustion, senescence, depletion, or inability to cope with environmental stressors. Shortening of telomere length has been linked to both senescence and cell death, further highlighting concerns related to aging and pathological stress. Therefore, stem cells derived from patients with advanced biological age and severe concurrent clinical features will require rejuvenation to reverse these deleterious effects of aging and disease. One means to bypass concerns with using aged autologous stem cells is to use young allogeneic stem cells. However, allogeneic stem cell use is complicated by multiple factors, including graft versus host rejection, infection, and arrhythmias, leading to poor survival and negligible persistence or engraftment. Therefore, multiple additional considerations will need to be addressed if allogeneic stem cells are to be used as a viable therapeutic solution to improving heart function and reversing heart disease and failure.

For adult stem cells to become a commercially available therapeutic product and treatment, the management and resolution regarding the uncertainty of their use as a therapeutic remains a key element. Adult stem cells have been in clinical testing since the early 2000s, and multiple strengths, such as the reduction of scar tissue and improvement of cardiac output, demonstrate a potential for these cells to have a beneficial effect. However, several concerns remain, including efficiency of the cells, understanding mechanisms responsible for therapeutic benefits, and understanding limitations for using stem cells as a therapeutic product and treatment. A Strengths, Weaknesses, Opportunities, and Threats (SWOT) analysis shows benefits and concerns related to the use of adult stem cells, collectively, as a cellular therapy to treat heart disease (see Figure 2). In the SWOT analysis, internal strengths and opportunities for the treatment and elimination of HF.

In assessing the therapeutic benefit of adult stem cells to treat CVD, factors influencing outcome can be categorized
into the stem cell attributes and function, the response of the endogenous tissue and cells post injection of the stem cells, and the external influences on the system, such as the patient and the procedure (see Figure 3). Factors influencing stem cell function include age of the cells, persistence and survival of cells, proliferation and commitment of cells for long-term benefit, and variable homogeneity of the cells. Endogenous influences may include location of the injection, engraftment of the injected cells, cell–cell communication of the injected cells to each other and to endogenous cells, and immune responses resulting from cell injection. External influences on therapeutic efficacy include severity of disease, patient age and recovery capacity, timing treatment with respect to disease onset and method of delivery, cell type and quantity used, and manufacturing of the cell. Cell type plays one of the most critical roles in therapeutic success, and cell biomanufacturing requires care with regard to oversight, planning, and implementation to be successful on a commercial level.\textsuperscript{32–35} With several influencing factors all unique to the individual patient in dictating the success of the therapeutic treatment, use of stem cells as a therapeutic remains challenging, but possesses the potential to cure heart disease rather than merely maintain function of a diseased cardiac function. As cell type is the primary factor influencing the potential therapeutic benefit, a clear understanding of various candidate cellular treatments is essential. A SWOT analysis for these individual therapies is summarized (Figure 4) and discussed in the next section.

**Figure 2. Strengths, Weaknesses, Opportunities, and Threats (SWOT) analysis of adult stem cells as a cardiovascular therapy.** The analysis is an overall snapshot of the use of adult stem cells in the marketplace in the treatment and cure for cardiovascular disease. The analysis looks at both the internal workings of the field, as well as the external origin, in terms of both helpful and harmful elements.

**Figure 3. Factors influencing therapeutic outcomes when using stem cells.** Factors influencing the therapeutic benefit from stem cells to treat cardiovascular disease can be broken down into 3 main categories of (1) the individual cell’s function, (2) the surrounding tissue function in response to the therapeutic cell treatment, and (3) the external influences on the cellular and tissue response toward repair and regeneration.
Current Status of Adult Stem Cell Therapies

**Bone Marrow–Derived/Mesenchymal**

Bone marrow–derived stem cells are composed of a diverse cell population, including mononuclear cells,26 MSCs,37 hematopoietic stem cells,38 side population cells,39 and small embryonic-like stem cells.40–43 With this diverse population of stem cells originating in bone marrow, a wide variety of approaches have been implemented for isolation and expansion,44–46 as well as variation in number of cells injected, means of delivery, delivery post injury, type of injury, and means of follow-up screening.47–50 Meta-analysis of bone marrow–derived stem cells therapy from multiple clinical trials47–53 concludes that the impact is beneficial, albeit modest with a 2% to 5% ejection fraction improvement in the first 3 months from bone marrow–derived stem cells in patients with acute myocardial infarction (MI) as well as chronic ischemic heart disease, despite variation in both therapeutic approach and means of analysis for results of the therapy.

Bone marrow mononuclear stem cells appeared as the first stem cell–based clinical trial cell therapy for MI between 2002 and 200554 with numerous studies initiated thereafter. In one study, improvement in global and regional myocardial function in the short term (6 months) was reported,55 whereas in the long term (18 months), a mixed result was reported with no significant differences in ejection fraction or improvement in diastolic function.56 In another study using mononuclear bone marrow stem cells, no significant functional changes between 3 and 6 months after injection in patients was found.57 These early clinical trial results cast doubts as to whether there were any beneficial effects of bone marrow therapy for MI because of fleeting positive impact on heart function long-term.

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### Figure 4. Strengths, Weaknesses, Opportunities, and Threats (SWOT) analysis of individual adult stem cells as a cardiovascular therapy

The analysis focus is the individual adult stem cell therapeutic treatment options. The analysis focuses on the internal workings of the individual treatment options based on current clinical trial results and ongoing preclinical research.

<table>
<thead>
<tr>
<th>System</th>
<th>Strengths</th>
<th>Weaknesses</th>
<th>Opportunities</th>
<th>Threats</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bone Marrow–derived/Mesenchymal</td>
<td>-Autologous -Readily procured -High Clinical Use -Allogeneic potential -Consistent modest Improvement in clinic</td>
<td>-Modulate immune response -Limited efficacy -Low survival, commitment and persistence -Consistent modest Improvement in clinic</td>
<td>-Well established isolation and expansion protocols -Excellent safety record</td>
<td>-Inconsistent clinical protocols for cellular isolation, processing, injection quantities and assessing outcomes</td>
</tr>
<tr>
<td>Cardiac Stem Cell</td>
<td>-Autologous -Endogenously located in the heart -Only stem cell demonstrated to derive into cardiac muscle</td>
<td>-Low endogenous population -Limited proliferation and durability -Efficiency of commitment -Patient variability</td>
<td>-Safety, Efficacy -Established isolation and expansion protocol -Potential to modify, enhance, and use in combinatorial therapy</td>
<td>-Misrepresentations and misinterpretations of therapeutic potential from controversial lineage tracing studies</td>
</tr>
<tr>
<td>Enhanced Stem Cell</td>
<td>-Autologous -Readily procured -Promising preclinical results in small and large animal models</td>
<td>-Understanding molecular mechanisms mediating enhancement of cells -Altered biological properties</td>
<td>-Provision for sustained cardiac functional improvement -Rejuvenation and restoration of regenerative potential -Creating novel biological phenotypes</td>
<td>-Modifications require additional time, money -Next generation of cellular heart therapy -Safety concerns following modification</td>
</tr>
<tr>
<td>Combination of MSC/CSC</td>
<td>-Autologous with allogeneic potential -Combination of cells may produce better results than single cell</td>
<td>-Mechanisms behind improvement poorly understood -Cell combinations introduce variability for determining ratios and assessing relative persistence</td>
<td>-Beginning clinical use -Allogeneic potential -Increasing complexity may possess greater relevance to biological mechanism of repair</td>
<td>-Combinations may not provide highly significant improvements compared to each separate cell type -Enhanced cells may provide comparable or superior benefit without biological co-dependence complexity</td>
</tr>
<tr>
<td>Cardiosphere-derived</td>
<td>-Autologous and allogeneic applications -Readily procured -Mixed cell population</td>
<td>-Heterogeneous cell types not well described -Functional efficacy negligible -Poor survival, commitment or persistence</td>
<td>-Unique culture environment -Mechanism of action may be in secretome rather than cells per se</td>
<td>-Combined or enhanced cell therapy providing functional benefit -Secretomes from other cell types</td>
</tr>
<tr>
<td>Secretomes</td>
<td>-Autologous and allogeneic potential -Readily procured -Present in multiple cell types</td>
<td>-Mechanisms behind functional Improvement not understood -No cells introduced, so depends upon endogenous repair</td>
<td>-Clinical use potential - Constituents could be fractionated and purified</td>
<td>-Sustained functional Improvement unknown -In vitro secretomes may not represent in vivo</td>
</tr>
</tbody>
</table>
and wide-ranging efficacy,68 prompting a need for additional preclinical studies focused on these and other stem cell treatment options.59 One of the recent studies involves swine cortical bone stem cells, demonstrating increased proliferation, migration, cardiac lineage commitment, functional gap junctions, and differential response to ATP and histamine stimuli as compared with MSCs or cardiac-derived stem cells.60

As an enriched subpopulation of bone marrow–derived cells, MSCs provide functional improvement in cardiac function, both in basic research and in clinical trials. MSCs were first analyzed in a clinical trial for acute MI using an intravenous cell injection method in a dose-dependent study, which provided pivotal safety and efficacy data.61 In the clinical trial Prospective Randomized Study of Mesenchymal Stem Cell Therapy in Patients Undergoing Cardiac Surgery (PROMETHEUS; clinicaltrials.gov: NCT00587990), 2 or 20 MSCs or a placebo were used to determine therapeutic response to HF after a heart attack. After 9 of 45 patients were enrolled, the trial was suspended because of slow accrual. Six patients received MSCs (2 low dose and 4 high dose; no placebo patients), and at the 18-month follow-up, findings suggested increased ejection fraction, decreased scar mass, and overall contractile improvement after MSC treatment compared with baseline of these patients.61 Additional clinical trials using MSCs include Phase I/III, 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 Nonischemic Dilated Cardiomyopathy (POSEIDON-DCM; clinicaltrials.gov: NCT01392625) and The Transendocardial Stem Cell Injection Delivery Effects of Neomyogenesis Study (TRIDENT; clinicaltrials.gov: NCT02013674). Interestingly, from the POSEIDON-DCM study and the TRIDENT study, research was performed to compare endothelial progenitor cells function after either allogeneic or autologous MSC administration in patients with HF; with results indicating that allogeneic MSC administration enhances proliferation of functional endothelial progenitor cells and improved vascular reactivity.65 Additional studies found MSC injections stimulated growth of endogenous CPCs and CSCs through cell–cell communication65,66 and paracrine signaling,65,66 but did not demonstrate persistence to engraft in the endogenous tissue after a month.67 A recent study has also analyzed functional effects of autologous MSCs compared with autologous cardiac-derived stem cell in a feline model after isoproterenol-induced cardiomyopathy.68 The beneficial effects of MSCs have more recently been explained through the exocytosis of secretome, composed of cytokines, growth factors, paracrine factors, microRNAs, and exosomes, to protect intact tissue, prevent additional damage, and support the endogenous repair of damaged tissue.69–72

Cardiac Progenitor/Stem Cells
Myocardial repair, regeneration, and functional processes in the heart are supported by CPCs localized within specific cardiac niches.73 Intrinsic and extrinsic factors regulate CPC turnover within the niche,74 thus affecting the potential for CPC reparability in response to myocardial injury.74 Accumulation of age-related changes,75 such as DNA damage, telomere attrition,76,77 epigenetic dysregulation, and environmental stress, impair CPC function.78 Heart-related pathologies primarily occur in the aged population,79 along with a compromised repair capability by the endogenous CPC pool. Senescent CPCs have limited capacity to expand and generate de novo cardiomyocytes, resulting in diminished cell turnover and acceleration of myocardial aging.80 Indeed, CPCs isolated from multiple patients exhibit variable growth kinetics, telomere length, and expression of cell cycle regulators.81,82 In addition to effects of chronological age, disease pathogenesis as well as combined genetic and environmental factors also impact CPC function. Analysis of patient characteristics and their respective CPCs revealed fast-, medium-, and slow-growing CPCs with growth rates inversely related to expression of senescent markers.83 Human CPCs represent an attractive target cell population to use in regenerative cell therapy, yet all samples from patients with HF fell short of exhibiting phenotypic characteristics comparable with fetal human CPCs, which are used as the gold standard for a healthy cardiogenic cell.82 Nevertheless, small animal in vivo studies demonstrated regeneration of cardiac tissue post MI using human CPCs and improved cardiac performance,81,83 prompting the initiation of clinical trials.

The cardiac Stem Cell Infusion in Patients with Ischemic Cardiomyopathy (SCIPIO) trial in 2009 was the first CSC clinical trial to use autologous CSCs in a randomized controlled study (clinicaltrials.gov: NCT00474461). After the first year of the SCIPIO trial, 16 treatment group and 7 control group patients were pooled from a subpopulation of patients who underwent elective coronary artery bypass graft surgery post infarction and demonstrated a left ventricular ejection fraction (LVEF) <40% at 4 months after the intervention.84 The right atrial appendage, resected during the operation, was used for isolation and expansion of the patient’s own CSCs. In total, 20 patients were treated with CSCs, and 13 patients served as the placebo group. In the treated group, 1 million autologous CSCs were infused via a balloon catheter into the graft vessel. Results indicated that the control group did not experience improved average LVEF between baseline, 12, and 24 months post treatment (30% [n=12], 32% [n=12], and 32% [n=5], respectively), whereas the CSC-treated patients experienced an average LVEF from 30% at baseline (n=12) to 38% (n=17) at year 1 and 42% by year 2 (n=12) post injection.84,85 The overall improved change in ejection fraction in the treatment group between injection and the 2-year follow-up was 12%, compared with the placebo group of 3.7%.85 Additionally, the infarct size decreased by 30% in the first year for treated patients,84 and the New York Heart Association cardiac functional classification in patients was both downgraded from baseline to 1-year after injection and sustained 2-years after injection.85,86 This trial was one of the most successful trials to date regarding functional improvement, over the long-term, along with structural improvement of the myocardium87 and patient quality of life based on the Minnesota Living with Heart Failure (MLHF) Questionnaire score. This study warrants additional in-depth clinical assessment through additional studies. It also prompted an introduction to a combinatorial therapeutic approach through use of both MSCs with CPCs.
Cardiosphere-Derived Cells found in the heart capable of forming spherical clusters in vitro (cardiospheres-derived cells [CDCs]) have also been studied for regenerative capacity. In the Cardiosphere-Derived Autologous StemCells to Reverse Ventricular Dysfunction (CADUCEUS) trial (clinicaltrials.gov: NCT00893360), patients with ischemic heart disease, percutaneous coronary revascularization, LVEF dysfunction (mean baseline <39%), and a MI that is 2 to 4 weeks old were included in the patient population. CDCs were obtained from each patient through endomyocardial biopsy and were then cultured between 1 and 2 months after the biopsy until ≤25 million cells were obtained and reintroduced to each patient. Scar size between placebo and treatment group from baseline to 6 months and 12 months is reported as unchanged for the placebo group (n=8) and a decrease from baseline to 6 months as 7.7% and baseline to 12 months as 12.3% for the treatment group (n=17). Additionally, regional systolic wall thickening was reported, and regional contractility increased at the 6 months follow-up between treated versus nontreated patients. However, unlike the SCIPIO study, functional parameters of cardiac hemodynamics, including left ventricular ejection fraction, end-diastolic volume, and end-systolic volume, did not improve compared with untreated patients. Furthermore, New York Heart Association classification was unchanged between the treated patient population and the untreated population, although MLHF score decreased in treated patients relative to untreated patients, with increased walking distance during the 6-minute walk test and peak oxygen consumption. Importantly, these data indicate that there are distinguishable differences between CPCs and CDCs, despite both originating in the heart. The comparatively homogeneous CPC population may be better suited for persistence that results in long-term functional cardiac improvement of the heart, whereas heterogeneous CDCs rapidly disappear from the myocardium and fail to confer long-term functional benefit. CADUCEUS results were likely also consequential to study design, including a limited patient population, the optimization of delivery method and cell dose, and the specific stem cell choice used in the study.

CDCs have found their way into multiple clinical trials, including (1) Autologous Human Cardiac-Derived Stem Cell To Treat Ischemic Cardiomyopathy (ALCADIA) where autologous CDC, from coronary artery bypass graft patients diagnosed with ischemic HF, were implanted back into the patients with a biodegradable gelatin–hydrogel-releasing basic fibroblast growth factor (clinicaltrials.gov: NCT00981006); (2) Transcoronary Infusion of Cardiac Progenitor Cells in Patients with Single Ventricle Physiology (TICAP) where patients with a single ventricular chamber were treated with autologous CDCs (clinicaltrials.gov: NCT01273857), and (3) Allogeneic Heart Stem Cells to Achieve Myocardial Regeneration (ALLSTAR), a phase I/II randomized double-blinded study using allogeneic CDCs (clinicaltrials.gov: NCT01458405). Results from ALCADIA for 5 of 6 treated patients (1 patient was excluded after acute occlusion of the graft 3 weeks after surgery) demonstrated mixed results (no placebo group reported), with one patient’s HF symptoms worsening, while the other 4 patients experiencing an average 9% (echocardiography) and 12% (magnetic resonance imaging) LVEF improvement between baseline and 6 months after treatment and an infarct size decrease by 3.3% of the total left ventricular volume. TICAP results from 14 infants (1.8±1.5 years) were found to have improved right ventricular ejection fraction by an average of 5.2% at 3 months post-treatment, and at 18 months, treated patients demonstrated higher right ventricular ejection fraction compared with nontreated patients (average 40.4% versus 31.5%). Results from the ALLSTAR trial are awaited.

Combination of Stem Cells Combining MSCs together with CPCs has evolved from earlier single cell trials with the rationale that a combination of cells possessing complementary effects will be better suited than any single cell type to provide enhanced functional and structural improvement within the failing heart. MSCs secrete cytokines to activate c-kit+ resident CPCs within the heart. A preclinical study using a mixture of 200 million MSCs together with 1 million CSCs in swine with MI demonstrated cardiac recovery to levels near baseline. LVEF, as well as left ventricular chamber dynamics, was improved in all treatment groups, and a 21.1% reduction in scar size was measured with the combinatorial therapy, as compared with a 10.4% reduction in CSC or 9.9% reduction using MSCs alone. A second preclinical study involving the combination of autologous MSCs and CPCs was performed in a porcine model of Gottingen swine 3 months after ischemia/reperfusion and received transendocardial injections of MSCs alone or a combination of MSC and cardiac-derived CSC compared with placebo. Treatments with either MSCs or the combination of MSC and CSC resulted in a significant reduction in scar size, increased viable tissue, improved wall motion, improved ejection fraction, and improved cardiac output 3 months after treatment. The synergistic effect of the dual cell therapy has recently been expanded into 2 scheduled clinical trials. One trial, Combination of Mesenchymal and C-kit+ Cardiac Stem Cells as Regenerative Therapy for Heart Failure (CONCERT-HF; clinicaltrials.gov: NCT02501811), is a randomized placebo-controlled phase II trial, where ischemic HF patients will receive either 150 million MSCs, 5 million CSC, a combination of 150 million MSC with 5 million CSC, or a placebo. The other is the clinical phase I/II trial Transendocardial Autologous Cells (hMSC or hBMC) in Ischemic Heart Failure Trial (TAC-HFT; clinicaltrials.gov: NCT02503280), where idiopathic DCM patients will receive autologous transplantation of either 200 million MSCs, a mixture of 199 million MSC with 1 million CSC, or a placebo. Both of these studies are in early implementation stages with study start dates of October 2015 and March 2020, respectively.

A recent variation of combinatorial cell therapy using MSC and CPC uses saphenous vein-derived pericytes (SVP) with CSCs. In a mouse model, researchers showed that combinatorial SVP/CSC treatment reduces infarct size and promotes vascular proliferation and arteriogenesis but does not demonstrate a greater contractility than that shown with the individual cellular treatments described earlier. Another combinatorial cell therapy approach involves the fusion of two stem cells.
to capture favorable attributes of each cell toward the genetic design and functional characteristics, resulting in improved repair and regeneration in pathophysiologic heart conditions. Fused cells have been identified to occur naturally in vivo, in particular with stem cells,99,100 and are, therefore, another potential approach to treating cardiomyopathies. Another next-generation combinatorial stem cells approach is to use CPCs, MSCs, and endothelial progenitor cells together into a cluster. This approach includes the use of endothelial progenitor cells, which are known to secrete autocrine, paracrine, and immunomodulatory factors that mediate cellular survival, persistence, and communication,101 as well as increase the vascularization within the cardiac tissue.102 The use of these 3 distinct stem cell populations and their respective cellular traits is purported to enhance endogenous repair within the heart for long-term improvement.103 This CardioCluster has already been shown to be efficacious in vitro, and small animal model studies are currently underway.

Enhanced Stem Cells

Findings of poor survival, engraftment, and persistence of adult stem cells in adoptive transfer therapy have prompted investigation into various enhancements to create a more optimized stem cell for cardiac regeneration. Stem cells can be enhanced through preconditioning, pharmacological intervention, and genetic modification. Preconditioning is the use of in vitro treatment of the stem cells with growth factors, hypoxic shock, or anti-aging compounds to improve cellular potency. Pharmacological intervention also enhances stem cell survival, engraftment, endurance, and commitment, similar to preconditioning techniques. Genetic modification enhances endurance, anti-apoptosis, survival, engraftment, and commitment of the cell. Use of these different approaches has been ongoing for both MSC and CPCs.

Preconditioning stem cells through multiple signaling pathways is advantageous because it simplifies treatment and because no genetic modification is needed. MSC factors used for preconditioning include vascular endothelial growth factor (VEGF),104 treatment with hypoxia105 to induce hypoxia-inducible factor 1α and stem cell–derived factor-1,106,107 fibroblast growth factor-2,108 hepatocyte growth factor,109 insulin growth factor,110 transforming growth factor-alpha.111 In CPCs, preconditioning with VEGF and stem cell–derived factor-1,112 connexin-43,113 hydrogen peroxide,114 hypoxia,115 and β-adrenergic signaling116 have all shown beneficial effects. Preconditioning serves as a means to enhance the cells toward favorable characteristics, but preconditioning results in a transiently altered state that does not benefit the cells long-term.

Pharmacological treatment of stem cells in vitro has shown improvement in phenotypic characteristics and potential therapeutic treatment during/after injection into tissue, enhancing activation, engraftment, commitment, and so on. Examples of in vitro pharmacological treatment include 5-azacytidine to demethylase DNA toward stem cell differentiation, as shown in MSCs117,118 and CPCs,119 as well as dexamethasone to induce stem cell differentiation, particularly for CPCs.120 Combinatory pharmacological and cellular treatment to improve stem cell properties include trimetazidine and MSCs to decrease fibrosis and improve myocardial recovery,121 simvastatin to increase systolic wall thickening and increase MSC engraftment,122 and catecholamines to stimulate proliferation of endogenous CPCs through the beta-2 adrenergic receptor signaling pathway.123

Genetic modification to enhance stem cell function has also been reported for several cells, including MSCs and CPCs. In MSCs, Bcl-2 has been engineered to activate survival pathways capable of suppressing hypoxia-induced apoptosis,124 VEGF, and angiotensin 1 for the promotion and formation of new blood vessels,125 survivin to increase cellular survival after introduction into the damaged tissue,126 and the stem cell homing factor stem cell–derived factor-1.127 In CPCs, genetic modified targets include nuclear protein kinase B (PKB), also known as Akt,128 protooncogene proviral integration site in Moloney murine leukemia virus (PIM-1),83,129,130 integrin-linked protein kinase (ILK),131 nucleostemin,132 notch,133 and β-adrenergic tolerance through 6-betaARKct.134 PIM-1, a prosurvival and proliferation gene kinase, has been used to enhance CPCs with demonstrated long-term engraftment, increased cardiac function, and reduced fibrotic scar as compared with regular CPCs or placebo in both small129 and large animal models.130 On a molecular level, a bifunctional genetically engineered fusion protein can be created to augment engraftment and binding of the functional stem cell(s) to damaged tissue, such as modification of αCD133-GPVI to bind CD133+ progenitor cells to damaged vessels producing higher capillary density and tissue vascularization.135 Through a variety of approaches, the use of genetic modification provides a means to amplify and enhance traits associated with therapeutic success. This advanced approach to cellular therapies may be applicable to the clinical therapies currently tried in nonmodified stem cells alone.

Secretome Byproducts

Collectively, the secretome is the entirety of byproduct secreted by a cell, parasite, or similar entity and includes both molecules that influence phenotypic behavior of cells and molecules signaling for inflammation, stress response, apoptosis, and similar responses.136 The secretome is composed of proteins, growth factors, cytokines, chemokines, microRNAs, and similar soluble factors, which together may mediate underlying MSC therapeutic benefit, despite lack of cellular engraftment. An early account of secretome released promoting beneficial effects was reported as a byproduct of MSCs genetically modified to overexpress AKT, which reduced infarct size and cardiomyocyte apoptosis in an adult rat model, as compared with controls of nonmodified MSC.66 Another early account of the beneficial byproduct effects of MSCs was reported from release of cytokines from bone marrow stem cells promoting new vessel formation, inhibiting cardiomyocyte apoptosis, and maintaining myocardial contractility.69

Beneficial growth factors secreted by MSCs include VEGF, transforming growth factor-β, secreted frizzle-related protein-1 and -2.137 Growth factors secreted from MSCs, such as VEGF, endothelin, epiregulin, Smad-5, secreted frizzle-related protein-1 and -4, and galectin-3, have also been reported to improve myocardial function, reduce cardiomyocyte apoptosis, reduce fibrosis in the infarct zone, and preserve myocardium after 7 days when injected into swine with acute MI.138
Likewise, MSC secretome with mobilizing factors hepatocyte growth factor, LIF, stem cell–derived factor-1, Skp1-Cull1-F-box-protein (SCF) and vascular endothelial cadherin (VE-cadherin) was purported to increase mobilization and homing of endogenous MSCs in adult DCM hamsters.\(^{139}\) Numerous microRNAs have been identified to modulate cardiac repair and regeneration,\(^{140,141}\) and these microparticles have also been identified as immature mRNA when secreted from MSCs.\(^{142}\) Additional contents of MSC exosomes include CD81, CD9, and Alix, which reduced infarct after injection in an ischemia/reperfusion injury mouse model.\(^{143}\)

CPCs also secrete exosomes and similar by-products that promote beneficial effects in the cardiac microenvironment to maintain and promote myocardial repair. Exosome-like vesicles containing multivesicular bodies were recently shown to be present in CPCs by ultrastructure examination.\(^{144}\) CPC exosomes contain miRNAs, such as miR-451, and can reduce cardiomyocyte apoptosis by over 50% when injected in an acute mouse myocardial ischemia/reperfusion model.\(^{145}\) Other microRNAs recently identified in the CPC secretome under hypoxic conditions include miR-292, miR-210, miR-103, miR-17, miR-199a, miR-20a, and miR-15b\(^{146}\); microRNA exosomes generated after 12 hours under hypoxic conditions and injected demonstrate improved fractional shortening and reduced fibrosis in an adult rat ischemia/reperfusion model.\(^{145}\) Likewise, microRNA 133a improves cardiac function through reduction of fibrosis and hypertrophy, as well as increasing vascularization and cardiomyocyte proliferation in a rat MI model.\(^{147}\) Despite this relatively recent flurry of excitement regarding exosome-mediated effects, the mechanistic basis remains poorly understood, and the long-term benefits in terms of restoring myocardial structure and function remain to be assessed. It is hypothesized that beneficial secretome is up-regulated, promoting molecular reprogramming in surrounding cells and leading to the beneficial effects. This may also explain how functional benefits may be found despite the lack of therapeutic cells present. Future studies of the stem cell secretome may help to define necessary product(s) that may contribute to prevention of heart disease, maintenance of heart function, and regeneration of healthy tissue to replace damaged heart myocardium.

**Future Directions**

The future of stem cell therapies is bright because it embodies a revolutionary form of medicine to treat disease with the potential to regenerate tissue. For cardiac therapies, adult stem cells represent the majority of cells currently under investigation for clinical trials with a more realistic potential to regulate, enhance, and utilize before and after reintroduction. Autologous stem cells have safety advantages because the cells obtained for the therapeutic treatment originate from the patient, demonstrating use for personalized medicine. Allogeneic therapies have demonstrated a mixture of success and failure within the patient because these cells do not originate from the patient nor maintain long-term engraftment; successes with these cells may be a result of the secretome released because more recent research is demonstrating a similar effect in viable tissue from just exosome and growth factor introduction as compared with allogeneic therapies. A major unresolved issue remains the dependency of allogeneic or secretome-based therapies on recruitment and activation of endogenous responses to mediate regeneration. Patient populations most likely targeted for cell therapy will be predominantly elderly or those with comorbidities (eg, diabetes mellitus, hypertension, smoking, obesity, etc) that compromise stem cell activity. If the efficacy of repair hinges on recruitment of endogenous repair, then research will inevitably need to address and augment rehabilitation and rejuvenation of impaired endogenous stem cells. Importantly, little attention is being paid to the relationship between endogenous stem cell competency in the clinical patient population to reparative potential, which is likely to be different from typical experimental animal models that are relatively young and overtly healthy. So too, emerging realities of current interventional approaches that offer positive structural remodeling in the absence of functional output improvement need to be acknowledged, as well as the unresolved durability of repair based on a single treatment intended to mediate repair for months or even years after delivery. These future frontiers will require even more innovative and creative solutions to truly deliver upon the promise of restoring myocardial performance with stem cell therapy. Positive results of adult stem cell–based therapy will most likely continue to be revealed in both basic science and clinical trials through increasingly effective, consistently safe, and predictably small but importantly progressive steps.

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**Disclosures**

M.A. Sussman is a founder and co-owner of CardioCreate Inc. K.M. Broughton has a modest interest in CardioCreate Inc.

**References**


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In the article by Broughton KM and Sussman MA, “Empowering Adult Stem Cells for Myocardial Regeneration V2.0: Success in Small Steps,” that appeared in the March 4, 2016 issue of the journal (Circ Res. 2016;118:867–880. DOI: 10.1161/CIRCRESAHA.115.305227), a correction was needed.

On page 869, a couple of sentences were revised as follows: “In New York, the New York Stem Cell Foundation, founded in 2005 for basic and translational scientific research related to stem cell biology, is a 501(c)(3) nonprofit organization and funded through private philanthropy.21 The state publicly allocates funds through the Empire State Stem Cell Trust Fund to the New York State Stem Cell Science Program with an initial 2007–08 appropriation of $100 million with an additional $50 million per year for 10 years.22”

This correction has been made to the current online version of the article, which is available at http://circres.ahajournals.org/content/118/5/867.full