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Stem and Progenitor Cell Therapies for Cardiovascular Disease

Christopher Sequeira[1], Ki Park[2], Carl J. Pepine[2*] and Christopher R. Cogle[3*]

[1]*Department of Medicine, College of Medicine, University of Florida, Gainesville, USA*
[2]*Division of Cardiovascular Medicine, College of Medicine, University of Florida, Gainesville, USA*
[3]*Division of Hematology/Oncology, College of Medicine, University of Florida, Gainesville, USA*

Abstract

Adult stem and progenitor cells have shown reparative potential in pre-clinical models of ischemia and infarction. These discoveries in conjunction with increased incidence and prevalence of heart disease and few new classes of pharmacologic agents for cardiovascular disease have paved the way for numerous clinical trials of stem and progenitor cell therapy for acute myocardial infarction and congestive heart failure. Nearly all trials have demonstrated safety and feasibility of stem cell therapies for cardiovascular disease. Many have suggested that injected cells patient populations, result in improved clinical outcomes. The future of cell therapy for heart disease will involve questions pertaining to patient populations, timing of therapy, cell population utilized, imaging techniques to assess efficacy and methods of cell delivery.

Keywords: Bone marrow; Cell therapy; Cardiovascular disease; Vasculogenesis; Paracrine regulation; Cell delivery

Introduction

Coronary artery disease and heart failure affect approximately 17,600,000 and 5,800,000 individuals, respectively in the United States [1]. Although rapid percutaneous coronary intervention (PCI) and stenting have saved more individuals from acute myocardial infarction (AMI), this life-saving procedure has also resulted in a greater number of AMI patients surviving but with ventricular dysfunction. Conventional post-infarction medical management of heart failure, including the use of beta-blockers, angiotensin converting enzyme inhibitors (ACE-Is), angiotensin receptor blockers (ARBs), statins and risk factor reduction (e.g., smoking cessation), have led to significant reduction in morbidity and mortality for these patients. Despite these advances, there have been few new pharmacological developments in post-infarction treatment that have shown meaningful survival benefit. Additionally, the effectiveness of conventional post-infarction medical management has led to an increase in the burden of patients that suffer from symptoms of heart failure, a result of left ventricular wall remodeling [2]. Ventricular remodeling is a detrimental condition and refers to the structural changes seen in the myocardium such as fibrosis after AMI that results in dys-synchrony of normal ventricular contractions.

In the past 25 years, heart failure is a category of heart disease where prevalence, incidence, hospitalization rate, total burden of mortality, and costs have increased with an estimated $40 billion spent annually in the US for treatment of these patients [1]. Thus the global impact of heart failure has underscored the importance and urgency of investigating novel strategies to improve morbidity and mortality after myocardial ischemic events.

Several reports over the past decade have demonstrated the multipotent capacity of hematopoietic stem and progenitor cells (HSPCs) [3,4]. In particular, adult bone marrow-derived HSPCs exhibit functional hemangioblast activity – generating both blood and blood vessels [5,6]. Thus, it has been hypothesized that bone marrow cells can repair ischemic and infarcted tissues. Mechanisms for this repair are purported to include (1) vasculogenesis, (2) pro-angiogenesis, and (3) modulation of inflammation (especially in the AMI setting). Other stem and progenitor cell types have been proposed as candidates based on their ability to support revascularization and myocardiogenesis after infarct.

Recently, the use of stem and progenitor cells for cardiac repair have been put to clinical test. The aim of this review is to summarize recent trials, discuss various stemcell types and proposed mechanisms of action, examine various methods of stem cell delivery and consider future directions for this relatively new and promising approach.

Bone marrow cell therapy clinical studies

Acute Myocardial Infarction: Following acute myocardial infarction (AMI), signaling from the infarcted myocardium leads to rapid mobilization of angiogenic cells and endothelial progenitor cells (EPCs) [7], which are believed to aid in myocardial repair and re-establishment of vascular perfusion. Given evidence that adult hematopoietic stem and progenitor cells exhibit hemangioblast activity [5]– that is, capacity to produce both blood and blood vessels – it has been hypothesized that concentration of bone marrow cells and direct injection into the infarcted myocardium will lead to vascular reconstruction and improved post-infarct recovery. In addition, bone marrow-derived cells are believed to regulate inflammation in the infarcted myocardium which decreases further myocyte apoptosis, increases collagen expression thus limiting infarct size and potentially promotes myocyte regeneration. All of these proposed mechanisms are aimed at attenuating or reversing post-infarct remodeling. To test

***Corresponding authors:** Christopher R. Cogle, MD, Division of Hematology/Oncology, University of Florida, 1600 SW Archer Road, Box 100278, Gainesville, FL 32610-0278, USA, Tel: 352-273-7493; Fax: 352-273-5006; E-mail: c@ufl.edu

Carl J. Pepine, MD, Cardiovascular Medicine, University of Florida, 1600 SW Archer Road, Box 100277, Gainesville, FL 32610-0277, USA, E-mail: carl.pepine@medicine.ufl.edu

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this hypothesis clinically, several investigative teams have evaluated autologous bone marrow cell therapies in the acute infarct setting.

In the clinical study Transplantation of Progenitor Cells and Regeneration Enhancement in Acute Myocardial Infarction (TOPCARE-AMI) [8], subjects with ST elevation myocardial infarction (STEMI) were randomized 24 hours after AMI to receive either bone marrow derived mononuclear cells (BM-MNCs) or peripheral blood mononuclear cells (PB-MNCs). These ex vivo cells were cultured for 3 days in a media promoting production of endothelial cell differentiation [9]. A total of 59 patients were randomized to receive the cells via intracoronary route at an average of 4.3 days after percutaneous intervention (PCI). The methods of evaluation of efficacy were left ventricular (LV) angiography, dobutamine stress echocardiography, fluorodeoxyglucose positron emission tomography (FDG-PET) scan and cardiac magnetic resonance imaging (MRI). Follow-up at 4 months revealed significant improvement compared to baseline of left ventricular ejection fraction (LVEF) and end-systolic volumes with normalization of coronary flow reserve. There was no significant difference in improvement over baseline when comparing between the BM-MNC vs. PB-MNC endothelial cell groups. The results obtained by echocardiography were similarly positive at the 4-month mark with a significant decrease in wall motion abnormalities. Furthermore, FDG-PET scans at 4 months also showed significant improvement in cardiac viability with no difference between the BM-MNC and PB-MNC endothelial cell group. A one-year follow-up, cardiac MRI showed maintained improvement in global LV function compared to baseline [10].

In the study of Bone Marrow Transfer to Enhance ST-Elevation Infarct Regeneration (BOOST) [11], 60 patients were randomized to a control group versus intracoronary BM-MNC infusion. The infusion took place an average of 4.8 days after PCI. Cardiac MRI was performed before cell infusion and again at 6 months. The results revealed significant increases in global LVEF in the treatment group, which was mostly due to improvement in regional systolic wall motion rather than LV end-diastolic volumes. A 5-year follow-up however did not show an appreciable difference in LVEF in control versus treatment group. On critical analysis, it appeared that the control group LVEF improved slowly over time to the levels that the cell group achieved earlier. These results suggest that that intracoronary infusion of BM-MNCs leads to a more rapid improvement of LVEF after STEMI [12].

In the study of Reinfusion of Enriched Progenitor Cells and Infarct Remodeling in Acute Myocardial Infarction (REPAIR-AMI) [13], 204 patients were randomized to either intracoronary BM-MNCs or placebo. Infusion took place an average of 4.4 days after PCI. LVEF was quantified by LV angiography, which took place just before cell infusion and again at 4 months. Improvements in global LVEF were only seen in subjects receiving cells 5 days or more after AMI and the greatest benefit was seen in patients with lower LVEFs. When specifically analyzing regional LV contractility, greater improvements were seen in the cell therapy group than placebo group. The study also tracked adverse clinical outcomes such as repeat MI, re-hospitalization for symptoms of heart failure, need for repeat revascularization and death all of which were significantly reduced in the cell therapy group at 4 month and 1 year periods. A two-year follow-up assessed a subgroup of 59 participants with cardiac MRI. The results were consistent with previous data showing improved LVEF, less relative infarct size and increased regional contractility in the cell therapy group versus placebo. And again the aforementioned adverse clinical outcomes were lower in the cell group versus placebo at two years [14].

In the clinical Autologous Mononuclear Bone Marrow Cells in Acute Anterior Wall Myocardial Infarction (ASTAMI) [15], 100 patients with anterior wall AMI were randomized to either intracoronary BM-MNC infusion or control group. The control group received no placebo or sham procedure. Single-photon-emission computed tomography (SPECT) and echocardiography were obtained before cell therapy. Intracoronary BM-MNC infusion took place a mean of 6 days after PCI. Two to three weeks after AMI, cardiac MRI was obtained. This time point was selected to avoid overestimation of the extent of infarct due to tissue edema. SPECT, echocardiography and cardiac MRI obtained at 6 months did not reveal statistically significant improvements in LVEF, end-diastolic volume or infarct size in the treatment versus control groups. A three-year follow-up utilizing echocardiography, cardiac MRI and exercise capacity testing found the same results. However, exercise capacity testing did show improvement in exercise time in the treatment group than control group [16]. Differences between ASTAMI and previous trials such as REPAIR-AMI may be related to number of cells infused (REPAIR-AMI delivered three times more than ASTAMI), types of infarcts treated (ASTAMI recruited only subjects with anterior infarcts) and methods of cell processing (Lymphoprep versus X-Vivo) [17].

In the Finnish Stem Cell trial (FINCELL) [18], 80 patients were randomized to intracoronary BMC infusion versus intracoronary placebo infusion. Time from PCI to cell therapy or placebo was on average 3 days. Left ventricular angiogram and echocardiography were used to assess changes in global LVEF at baseline and 6 months. Intravascular ultrasound, holter monitoring, microvolt T-wave alterans during maximal exercise and signal averaged electrocardiogram were also performed at baseline and at the 6 month interval. At the 6-month endpoint, global LVEF as measured by echocardiography was significantly greater in the cell therapy group than placebo group [19]. Minimum lumen area of the stented vessel decreased in both treatment and placebo groups without significant differences between the two groups. The other parameters gathered through IVUS did not change significantly over the initial 6 month interval. Arrhythmia risk variables were also not significantly different between the two groups.

In the Myocardial Regeneration by Intracoronary Infusion of Selected Population of Stem Cells in Acute Myocardial Infarction study (REGENT) [20], 200 patients with anterior wall AMI and LVEF ≤ 40% were randomized in 2:2:1 fashion into three parallel groups: (1) CD34+CXCR4+ intracoronary infusion (selected), (2) BM-MNC intracoronary infusion (unselected), and (3) control group, which did not receive placebo or undergo sham collection procedure. Cells were administered at a mean of seven days after PCI. Left ventricular angiography done at the time of cell infusion and cardiac MRI done 1-3 days after cell infusion were performed as baseline measurement. Repeat measurements were assessed at 6 months. Modest increases (3%) in LVEF were detected in the selected and unselected cohorts; however, no change in LVEF was observed in the control cohort. Significant increases in LVEF was observed only in patients with severely depressed left ventricular function, as defined by LVEF <37%.

In the study of Clinical Benefit and Long-Term Outcome After Intracoronary Autologous Bone Marrow Cell Transplantation in Patients with Acute Myocardial Infarction (BALANCE) [21], 124 patients were randomized to either intracoronary BM-MNC infusion or control group. The control group did not receive placebo or undergo

sham collection procedure. BM-MNC infusion took place on average 7 days after reperfusion. Quantitative LV angiography was utilized to evaluated LV indices at baseline, 3 months, 12 months and 60 months. At 3 months, LV contractility indices were much improved in the treatment group compared to the control group. As well, results at 12 and 60 months revealed sustained improvement of LV performance in the treatment versus control group. There was also statistically significant mortality reduction in the treatment group.

Cao et al. [22] randomized 86 patients to intracoronary BM-MNC infusion or control groups. Intracoronary BMC took place 7 days after reperfusion. Follow-up evaluations were obtained with echocardiography, SPECT and coronary angiography at baseline, 6 months and 4 years. Echocardiography and SPECT results showed statistically significant improvement seen in the BM-MNC group in regards to LVEF and ESV over the control group at both time points though SPECT revealed no differences in infarct size at 4 years.

In a multicenter, randomized trial of intracoronary infusion of autologous mononuclear bone marrow cells or peripheral mononuclear blood cells after primary PCI (HEBE) [23], 200 patients were randomized to receive BM-MNC, PB-MNC or control group (no placebo, no sham). MNCs were isolated by Lymphoprep, similar to the ASTAMI trial. MNC infusion was delivered at a mean of 6 days after PCI. Cardiac MRI was performed at baseline and again at four months. Four month follow-up results revealed no statistically significant changes in global or regional left ventricular systolic function. One potential criticism may relate to the method of MNC separation; however, the HEBE investigators found excellent recovery and hematopoietic progenitor colony formation when using their methods of MNC isolation [24]. A post-hoc analysis suggested that patients with an initially dilated left ventricle benefited from cell therapy as it prevented further dilation.

Janssens et al. [25] randomized 67 patients to either BM-MNC or placebo groups. Cardiac MRI was performed at baseline (4 days after PCI) and 4 months. The study results showed increase in global LVEF and LV volumes in both groups but the differences were not statistically significant. Cardiac MRI showed greater reduction in infarct volume of patient receiving BM-MNCs, particularly in those with larger infarcts.

Chronic myocardial ischemia

In one of the first cell therapy trials for patients with ischemic cardiomyopathy, Perin et al. [26] conducted a nonrandomized study of 21 patients with chronic myocardial ischemia who received intramyocardial injection of BM-MNCversus control (no placebo, no sham). Inclusion criteria for ischemic heart failure included reversible defects detectable by SPECT, LVEF <40% and ineligibility for PCI or surgical revascularization. Left heart catheterization (LHC), electromechanical mapping (EMM)and SPECT imaging were obtained for baselines.Two month follow-up showed improvements in LVEF, symptoms of heart failure, symptoms of angina pectoris, and exercise indices in the treatment group. Subsequent four-month follow-up included LV angiogram and EMM,which showed statistically significant LVEF improvement from baseline with a reduction in end-systolic volume (ESV.) There was no difference in end-diastolic volumes (EDVs). As well, EMM showed significant improvement in mechanical function at the site of injection of BM-MNCs.

In a small case series, Smits et al. [27] treated five patients with intramyocardial injection of skeletal muscle myoblasts in a feasibility study. The 5 patients selected all had NYHA class>II, received optimal medical therapy, had LVEF between 20-45%, and had to be greater than four weeks after AMI. Muscle biopsy was performed from the quadriceps and myoblasts were sent isolated. As in the previous study EMM was used to target areas of treatment and the myoblasts were injected into the sites of electromechanical disassociation. Measurements of LVEF by LV angiography, nuclear scintigraphy and MRI at baseline, 3 months and 6 months showed statistically significant increases. Most importantly, this study provided the safety data needed for larger scale trials of autologous skeletal myoblasts in patients with ischemic cardiomyopathy.

Patel et al. [28] randomized 20 patients into either subepicardial transplant of BM-MNCs during coronary artery bypass grafting (CABG) or control groups.Patients selected had ischemic heart disease with an LVEF <35% and NYHA class III or IV heart failure. Off-pump coronary artery bypass were performed in both the treatment and control group but the treatment group also had a bone marrow harvest prior to the procedure. After completion of the CABG, pre-determined sites of myocardium with akinesis and dyskinesis by SPECT and echocardiography were injected with BMCs. At 6 months, the cell therapy group showed statistically significant improvement in NYHA function class compared to the control group. At 1, 3 and 6 month follow-up timepoints there was also a significant increase in LVEF realized.

Selection of cells and mechanisms of action

The mechanisms of action of bone marrow derived stem and progenitor cells in ischemic cardiac disease appear to be multi-factorial. There is evidence that a direct paracrine effect results in decreased cardiomyocyte apoptosis, recruitment of resident stem cells and an increase in cardiomyocyte proliferation [29] all of which cause an increase in myocyte number and subsequent benefits in terms of myocardial function. This same paracrine mechanism can account for an increase in neovascularization due to stem cell recruitment leading to increased oxygen delivery to damaged myocardium and consequently a decrease in heart failure and anginal symptoms. The selection of a particular cell type that holds the most therapeutic benefit is less clear and trials have employed BM-MNCs, endothelial progenitor cells (EPCs), multipotent mesenchymal stromal cells (MSCs) and skeletal myoblasts. Methods of obtaining these stem cells also vary in trials with some studies choosing to collect cells through peripheral blood after stimulation with growth factors and others tapping the rich reservoir of cells directly in the bone marrow. In fact, trials are underway to evaluate the utility of adipose derived hematopoietic, endothelial and mesenchymal cells for use in similar applications [30].

Endothelial progenitor cells

Endothelial progenitor cells (EPCs) have been reported to express CD34, CD133 and VEGFR2. Studies have shown that coronary artery disease patients have EPCs at reduced levels and with impaired migratory function [31]. Kawamoto et al. [32] showed that when radiolabeled human EPCs were injected intravenously into rats with induced myocardial ischemia, the radiolabeled cells accumulated at the focus of ischemia with incorporation in the new vasculature. Follow-up necropsy showed less left ventricular scarring and increased capillary density in the ischemic area. These studies support the rationale to expand, concentrate and administer EPCs into the ischemic myocardium. As there have been numerous reports re-defining EPCs based on cell surface expression, there are as many potential cardiovascular cell therapy trials.

Multipotent mesenchymal stromal cells

Multipotent mesynchymal stromal cells (MSCs), which express STRO1 but lack CD34 and CD133, are found in bone marrow and can be expanded ex vivo [33]. As the progenitor cell to cardiac myocytes, their regenerative utility is being extensively investigated. MSCs have been shown to differentiate into cardiac myocytes in vitro but at a slower rate in vivo [34]. Much like EPCs, they also exhibit paracrine activity by secreting bioactive factors that inhibit fibrosis, apoptosis and enhance angiogenesis [35]. Studies evaluating MSCs in cardiovascular disease have been small. But one randomized trial of 69 patients revealed improved left ventricular function three months after intracoronary infusion, indicating that this may be a promising therapy for humans [36]. Evidence from a recent mouse study of MSCs injected into the peri-infarct area after coronary artery ligation and in the hindlimbs of mice with diabetic neuropathy resulted in malignant sarcomas with myogenic differentiation in 30% of hearts and 46% of hindlimbs [37]. On karyotype analysis of the MSCs, abnormalities (fusion, fragmentation and ring formation) were found in passage 4 and passage 8 MSCs. No MSCs from passage 0-3 were injected and evaluated. These findings suggest that tumor forming murine MSCs develop chromosome abnormalities after forced ex vivo expansion, and serve as a cautionary note for MSC cell therapy.

Skeletal myoblasts

Skeletal myoblasts harvested from human muscle are capable of differentiating into myotubes but not cardiac myocytes [38]. One animal study also reported that the myotubes do not electrically integrate with surrounding cardiomyocytes and are actually hyperexcitable causing dysynchronous myocardial contraction [39]. Despite these findings, there have been multiple animal studies that have shown improved LV function after ischemic events [40]. These findings may be a result of the paracrine function of these cells rather than their differentiation to myotubules much like other progenitor cells utilized for cell therapy [41]. The same findings were unable to be duplicated in human models. Most prominently, the MAGIC trial employed direct injection of skeletal myoblasts into akinetic tissue during CABG procedures [42]. The results at 6 months after intervention revealed no statistically significant increase in LVEF as evidenced by echocardiography.

Delivery methods

Although the ideal cell type for repair of cardiac dysfunction remains unclear, it has become apparent that the method of cell delivery is important to the success of these various therapies. Traditionally, studies have utilized either intracoronary or intramyocardial delivery methods (Figure 1). Perhaps the simplest and safest way to deliver cells is through intravenous infusion. However, early trials showed that these delivered cells failed to home to the myocardium and instead were sequestered in other organs such as the lungs, liver, spleen, kidneys, bladder, and femur [43]. To improve site specificity and cell retention, investigators have increasingly used catheter-based delivery systems for direct myocardial application. Intracoronary infusions utilize the same techniques as performed in traditional percutaneous coronary intervention (PCI). Cells are delivered through a catheter with concurrent balloon occlusion of the artery thus preventing wash out of cells. Although this allows better targeting of cells to infarcted myocardium and is relatively cost effective, this method is not without an increase in risk. Excess cell infusion can lead to additional cell volume which has been associated with increased coronary obstruction [44]. Also, it remains unclear how effective intracoronary infusion may

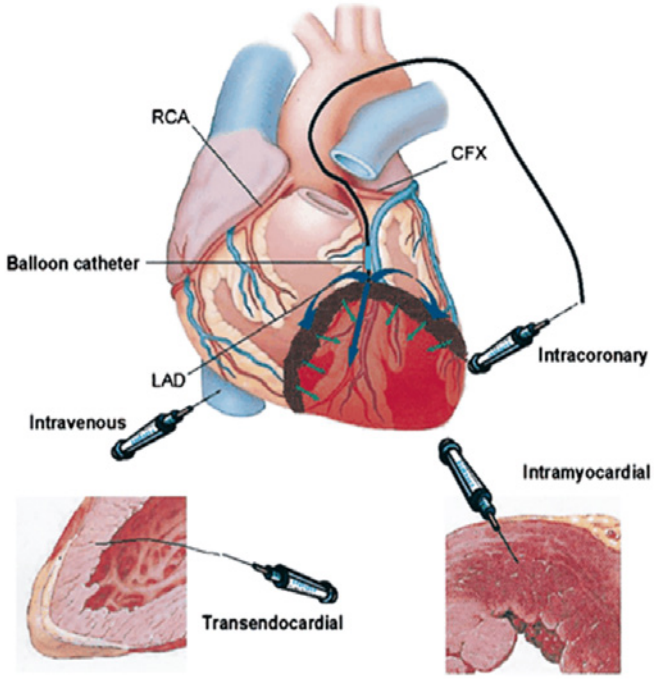

Figure 1: Various methods of stem cell therapy delivery [58] (RCA – right coronary artery, CFX – left circumflex artery, LAD – left anterior descending artery).

be in regards to homing and retention of cells within the myocardium with less than 10% retention in one prior study [45].

Transendocardial delivery technique injects cells directly into the myocardium at the peri-infarct area through guidance via an electromechanical mapping system. This technique is advantageous over intravenous and intracoronary methodologies as cells are directly injected into scarred myocardium with less cell attrition and avoids excess intracoronary infusion.

Accessing the venous system with the heart is another less utilized but potentially viable method. The coronary sinus approach has been utilized with various cell types as an alternate approach to accessing the myocardium. Advantages include simplicity, stable and direct access and accurate delivery into target myocardium.

A few studies have also evaluated the potential for non-catheter based direct injection of cells. One approach is to implant cells at the time of cardiac surgery when direct access is readily available. A few early clinical studies have tested bone marrow cell injection at the time of coronary artery bypass grafting (CABG). These studies demonstrated safety and feasibility of cell application after CABG; however, clinical benefit has yet to be proven [46,47]. Moreover, follow-up times in these early trials have ranged from only 2 weeks to 6 months. Controlled studies with longer follow-up are needed to determine clinical benefit.

Despite advancements being made in the arena of stem cell delivery to the heart, retention of stem cells within the myocardium remains a significant problem. One novel approach to this issue is the idea of developing a "scaffold" or cellular framework for the cells to reside in at the time of delivery. Synthetic polymers such as fibrin glue are already readily used in cardiac surgery as a procoagulant and could be

easily applied towards this new purpose [48]. These materials act as an adhesive to provide a structure for freshly implanted cells and combat cell leakage. Along with other methods such as a "patch" containing cells, these new methods hold significant promise.

Catheter types

Several different catheters for endocardial delivery of cells are currently being investigated in a variety of clinical trials. The majority of these catheters gain access to the left ventricle via retrograde access across the aortic valve in an approach similar to traditional left heart catheterization. One specific device, the Myostar (BDS)is an injection catheter used in conjunction with electromechanical mapping. The Myocath (Bioheart) catheter (Figure 2) is currently in development for delivery of myoblasts after LVAD implantation.

Two alternate devices, the Helix Classic (BioCardia) (Figure 3) and Stilleto (Figure 4) catheter (Boston Scientific) contain separate core and support catheter units. The Stilleto catheter is currently approved for use in peripheral vascular cases. These catheters do support insertion of the support catheter over a guide wire. In the Helix catheter, the injection catheter is helical based on pacemaker lead technology which improves stability of the needle tip during injection. The Stiletto injection tip is spring loaded and may provide more force to penetrate the myocardium in dense, fibrotic tissue.

Finally, TransAccess Delivery System (Medtronic) is also under investigation in cell therapy applications for patients with cardiovascular disease (Figure 5). This system provides access through the coronary venous system via epicardial approach. This system is unique in that an intravascular ultrasound (IVUS) probe is contained within the support catheter, thereby permitting direct visualization of the adjacent coronary artery. With IVUS guidance, the appropriately selected coronary vein is punctured allowing access to the myocardium for the injection catheter (Table 1).

Figure 2: Myocath by Bioheart is used to implant myoblasts after LVAD implantation [59].

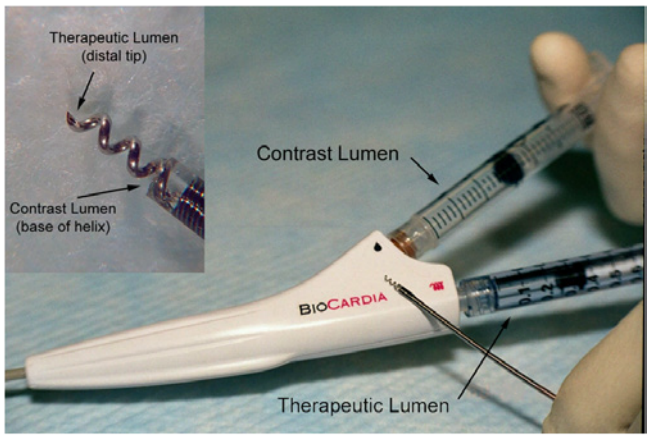

Figure 3: Helix Classic transendocardial delivery system [60].

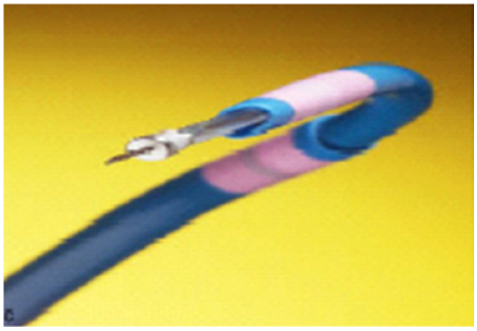

Figure 4: Stiletto™ catheter (Boston Scientific) [61].

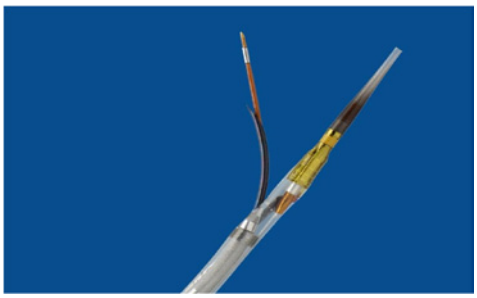

Figure 5: TransAccess epicardial injection system [62].

Future directions

As cell therapy for cardiovascular disease is still in its infancy, there are many advances that must be made in order for it to become a proven therapeutic option. There is a lack of consistency among the aforementioned trials in terms of timing of therapy, cell population utilized, imaging techniques to assess efficacy and methods of cell delivery. It is difficult to compare these studies against each other due to these confounding factors and future studies should be structured to resolve these inconsistencies.

Timing of therapy in the studies varied from 3 to 7 days after AMI but there has not been a definitive study that has determined the optimal time for therapy. A pooled subgroup analysis concluded that the optimal time of therapy may be between 4 to 7 days post AMI after review of 7 trials with 660 patients [49]. To prospectively test the effects of time of cell therapy in cardiovascular disease, the NIH NHLBI created the Cardiovascular Cell Therapy Research Network (CCTRN) in 2007 and is comprised of cardiovascular centers at the University of Florida, University of Minnesota, Cleveland Clinic, Vanderbilt University and Texas Heart Institute. The CCTRN initiated three clinical studies: TIME, LateTIME and FOCUS. In TIME, patients with STEMI and LVEF ≤ 45% are randomized to intracoronary BM-MNC injection either 3 days or 7 days after AMI [50]. In LateTIME, patients with STEMI and LVEF ≤ 45% are randomized to intracoronary BM-MNC injection at 14-21 days or placebo injection post-MI [51]. The BM-MNCs are isolated by Ficoll gradient using a closed-system,

Study	Cell type	Delivery route	Timing of infusion post-MI	Follow-up period	Follow-up method	Outcome
TOPCARE-AMI [8]	BM-MNCs	Intracoronary	4.3 days	4 months 1 year	LV angiography Dobutamine stress echocardiography FDG-PET	Improvement in LV function
BOOST[11]	BM-MNCs	Intracoronary	4.8 days	6 months 5 years	Cardiac MRI	Improvement in LVEF at 6 months but no appreciable increase in LVEF over 5 years
REPAIR-AMI[13]	BM-MNCs	Intracoronary	4.4 days	4 months 1 year 2 year	LV angiography Cardiac MRI	Greater improvements in LVEF in cell therapy patients vs. control. Less adverse clinical outcomes* in cell therapy group at 4 months, 1 years and 2 years
ASTAMI[15]	BM-MNCs	Intracoronary	6 days	6 months 3 year	SPECT Echocardiography Cardiac MRI Exercise capacity testing	6 month follow-up did not show statistically significant improvements in LVEF, EDSV or infarct size. However, 3 year follow-up revealed improvements in exercise time.
FINCELL[18]	BM-MNCs	Intracoronary	3 days	6 months	LV angiography Echocardiography	Statistically significant improvement in LVEF at 6 months.
REGENT[20]	BM-MNCs vs. CD34+CXCR4+ vs. placebo	Intracoronary	7 days	6 months	LV angiography Cardiac MRI	Modest increases in LVEF were realized in the cell therapy groups over placebo.
BALANCE[21]	BM-MNCs	Intracoronary	7 days	3 months 12 months 60 months	LV angiography	Improved LV contractility and mortality reduction over 3, 12 and 60 months.
Cao et al.[22]	BM-MNCs	Intracoronary	7 days	6 months 4 years	Echocardiography SPECT LV angiography	Statistically significant improvement in LVEF and ESV at both time periods.
HEBE[23]	BM-MNCs vs. PB-MNCs vs. control	Intracoronary	6 days	4 months	Cardiac MRI	No statistically significant changes in LVEF though a post-hoc analysis found that patietnts with initial dilation of the LV benefited as cell therapy prevented further dilation.
Janssens et al.[25]	BM-MNCs	Intracoronary	4 days	4 months	Cardiac MRI	Greater reduction of infarct volumes in cell therapy patients particularly those with larger infarcts. No statistically significant increase in LVEF or LV volumes.

Table 1: Summary of studies employing cell therapy after AMI.

automated unit (Sepax). Clinical outcome measures are evaluated by echocardiography and cardiac MRI. The purpose of FOCUS is to test the effects of intramyocardial BM-MNC injection in patients with ischemic cardiomyopathy (LVEF ≤ 45%) [52]. The BM-MNCs are isolated in the same manner as TIME and LateTIME and clinical outcomes are measured by echocardiography, SPECT imaging and exercise treadmill MVO2. The LateTIME and FOCUS trials have closed to accrual and follow-up evaluations are being performed. Together, these trials are anticipated to (i) define optimal time for administration of BM-MNCs and (ii) determine the impact of BM-MNCs in congestive heart failure (CHF).

The CCTRN has also created a Biorepository tasked with defining cell phenotype and function of the injected BM-MNCs [53]. In addition, the CCTRN Biorepository is charged with evaluating peripheral blood cell phenotype and function in an effort to measure cell therapy induced changes. The Biorepository Core also cryopreserves extra cells for future post-hoc analyses. Results from the Biorepository Core will provide mechanistic insights into the effects of autologous BM-MNC therapy for AMI and CHF.

As previously described, cell populations that provide the most benefit is also an intensely debated topic. Most of the trials we reviewed used BM-MNCs for therapy and few utilized selected cultured progenitor cells, MSCs and skeletal myoblasts. Unselected BM-MNCs are relatively easy to procure and were primarily used to show feasibility and safety. In the coming years, selection of certain stem and progenitor cell populations (CD34+, CD133+, CXCR4+, $ALDH^{bright}$) will be tested. As inducible pluripotent stem (iPS) cell technology improves and provides a certainty of safety, these cells are also likely to be administered in a cardiovascular indication for tissue repair. Increasingly popular and currently being studied are the potential of embryonic stem cells in cardiomyocyte regeneration. There have been favorable findings in primate studies but there is currently is not enough data to extrapolate these to human models [54].

Another strategy for optimizing cell type is ex vivo pre-treatment. For example, patients with diabetes mellitus are known to have defects in bone marrow cell function [55]. Ex vivo treatment of impaired cells has resulted in reversal of defects and return of normal function. Therefore to enhance the reparative potential of autologous BM-MNCs from patients with diabetes, treatment of the cells prior to delivery into the heart may improve clinical outcomes. These ex vivo enhancement techniques represent a novel strategy to personalize cell therapy in the future.

There have been a number of imaging techniques employed for follow-up evaluations including SPECT, PET and cardiac MRI. Multiple studies have recently reported the superiority of cardiac MRI over PET and SPECT in detecting perfusional defects and quantifying scar tissue [56]. Yet to be defined are best methods to measure response to cell therapy injections: infarct size, regional wall motion, regional ejection fraction, global ejection fraction.

Methods of cell delivery have also widely varied in the studies reviewed. Some of the studies utilized subepicardial injections during CABG while most of the others simply employed intracoronary infusions or transendocardial intramyocardial injection of cells. The goal of cell delivery is to administer the cells so that location accuracy and cell retention are optimized. The mode of delivery which provides the most benefit is not clearly defined but is thought to be equally as

important as the cell type chosen. There have not been any head-to-head trials between the differing methods. It is clear that most studies aim to be as non-invasive as possible which is why intracoronary cell infusion has been more common than surgical approaches. Needless to say, the most effective delivery method needs further investigation, and will likely depend on the intent of cell application: revascularization versus mitigation of inflammatory response versus myocyte transdifferentiation.

Although initial cell therapy trials have focused on patients with either AMI or CHF, several other patient populations could benefit from these newly developed methods. Patients with peripheral arterial disease (PAD) are perhaps the most logical extension of studies focusing on angiogenesis. Treatment options for PAD are similar for traditional CAD involving PCI and medical therapy. However, restenosis is common and patients often remain severely debilitated due to their condition. As the pathophysiology of PAD may be comparable to CAD, it has been theorized that cell therapy may be a viable option for PAD as well. Development of improved catheter delivery systems has been a vital step in realizing these ideas. Various catheter techniques and devices have been designed for delivery to minimize damage to cells from shear forces as well as maximize exposure time of the cells to the targeted vessel.

Left ventricular assist devices (LVADs) are an increasing part of therapy for chronic ischemic heart failure. For many patients in whom transplant is not an option, these implanted devices serve as a necessary means to survive. However, patients are unable to be weaned off these devices secondary to continued adverse remodeling of the left ventricle. In patients with ischemic cardiomyopathy, restoration of viable myocardium could play an important role in restoring LV function in patients with LVADs. A hybrid approach of using stem cell therapy in patients with chronic ischemic cardiomyopathy (ICM) requiring LVAD support has been tested [57]. In one small study, a mix of progenitor cells was administered directly to areas of hibernating myocardium. This study demonstrated improved myocardial perfusion by nuclear imaging. In theory, use of LVAD therapy can serve as a temporizing measure to unload the left ventricle while cell therapy may improve long term function through angiogenesis.

Although the majority of current stem cells trials have focused on patients with ST elevation myocardial infarction (STEMI), the largest majority of patients admitted with an acute coronary syndrome (ACS) suffer from non-ST elevation myocardial infarction (NSTEMI). Increasing evidence demonstrates that NSTEMI patients have similar if not higher risk of long term adverse cardiovascular events when compared to STEMI patients. To our knowledge, no cell therapy studies have been conducted in this population and provides an ideal opportunity to improve outcomes in a large population of patients with cardiovascular disease.

With so many unanswered questions, the future of cell therapy for cardiovascular diseases holds many opportunities for defining clinical studies.

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