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Combined Usage of Stem Cells in End-Stage Heart Failure Therapies

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ABSTRACT

Remarkable achievements have been made in the clinical application of mechanical circulatory support and cardiac transplantation for patients with end-stage heart failure. Despite the successes, complications associated with these therapies continue to drive cardiac regenerative research utilizing stem cell based therapies. Multiple stem cell lineages hold clinical promise for cardiac regeneration—mostly through cellular differentiation, cellular fusion, and paracrine signaling mechanisms. Bone marrow-derived endothelial progenitor cells are among the most intriguing and controversial cell types currently being investigated. Formidable barriers exist, however, in finding the ideal cardiac regenerative stem cell, such as identifying specific lineage markers, optimizing in vitro cellular expansion and improving methods of stem cell delivery. Hybrid approaches of cardiac regeneration using stem cell therapies in conjunction with immunomodulation after cardiac transplantation or with mechanical circulatory support produce cutting edge stem cell technologies. This review summarizes the current knowledge and therapeutic applications of stem cells in patients with end-stage heart failure, including stem cell therapy after implantation of mechanical circulatory support and cardiac transplantation. J. Cell. Biochem. 115: 1217–1224, 2014.

KEY WORDS: HEART FAILURE; STEM CELL; HEART TRANSPLANTATION; LVAD

The therapeutic potential of stem cells for cardiac regeneration has been intensely studied for nearly a decade. Significant advances in stem cell biology, including a better understanding of the mechanisms of stem cell plasticity and differentiation, have paralleled the evolving animal and human clinical trials. Here we briefly introduce stem cell-based cardiac therapies, followed by a discussion of the limitations of stem cell therapies in treating end stage heart failure (HF).

The self-renewal ability and differentiation potential of pluripotent stem cells make them a valuable source for producing transplantable cardiomyocytes. Embryonic stem cell (ESC)-derived cardiomyocytes and adult stem cells derived from bone marrow (BM), adipose tissue, and cardiac locations have been used for myocardial regeneration. ESC-derived cardiomyocytes have been shown to improve myocardial function in animal models with experimentally induced myocardial infarction (MI) [Laflamme et al., 2007; van Laake et al., 2007]. Intracoronary injection of BM-derived cells in patients with chronic HF has been shown to result in a 30% reduction in infarct size and a corresponding 15% improvement in ejection fraction (EF) [Strauer et al., 2005]. Direct myocardial injection of $CD133^+$ BM stem cells into infarct border zones has led to a significant improvement in left ventricle EF (LVEF) from 37% to 47% [Stamm et al., 2007]. Intracoronary infusion of adipose-derived stem cells (ADSCs) within hours after percutaneous revascularization in patients presenting acute MI improved LVEF with reduced scar formation [Houtgraaf, 2012]. Cardiac stem cells (CSCs) have been shown to differentiate into cardiac, smooth muscle, and endothelial cells (EC) [Beltrami et al., 2003]. Infusion of autologous Lin^-/c-kit^+ CSCs into patients with post-infarction LV dysfunction improved LVEF from 30.3% to 38.5% [Bolli et al., 2011].

Despite the progress in restoring heart function, predominantly after acute MI, the application of stem cells in end-stage HF remains limited. Current proven strategies for treating MI are based on replacing (heart transplantation and total artificial heart) or

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supporting (left ventricular assist devices [LVAD], external device therapies, etc.) the failing heart [Stehlik et al., 2012; Kirklin et al., 2013]. There remains significant concerns in regard to the long-term efficacy of these treatment strategies. For example, heart transplantation is complicated by the development of malignancy, infection, rejection, and transplant vasculopathy [Stehlik et al., 2012]. Similarly, implantation of a long-term mechanical circulatory device may lead to coagulation abnormalities, infection, and right ventricular dysfunction [Kirklin et al., 2013]. These complications continue to drive interest in using stem cell therapies as a means to restore myocardial function. However, as an isolated strategy, the promise of stem cell therapeutics remains largely unrealized. Critical gaps remain in the understanding of the basic mechanisms involved in stem cell therapeutics, including methods of harvesting, isolating, and inducing stem cell differentiation. Additionally, in vivo strategies to optimize engraftment, improve mobilization, and enhance survival of stem cells will be important to make widespread clinical application a reality [Mummery et al., 2010; Cashman et al., 2013; Sanganalmath and Bolli, 2013].

In spite of these formidable challenges, the potential of stem cell therapeutics for HF remains enticing, especially in combination with currently available advanced HF treatment strategies. For example, progressive myocyte loss from chronic uncorrected ischemia, inflammation, and apoptosis, which results in fibrosis and replacement of viable myocardium with scar formation, could be reversed while patients are supported with LVAD therapy [Birks, 2010]. Cardiac cell-based therapies could also be used to monitor clinical phenomena such as chronic rejection, or utilized to modify immunologic responses after heart transplantation and directly halt progression of chronic vasculopathy. In this manuscript, we provide an updated and innovative review of the current knowledge and therapeutic applications of stem cell therapy in patients with advanced HF, including heart transplantation and LVAD. Finally, we present promising advancements for hybrid approaches utilizing stem cells and mechanical circulatory support device therapies.

IMPLICATIONS OF STEMS CELLS IN TRANSPLANT ALLOGRAFT VASCULOPATHY

Transplant vasculopathy (TV) is characterized pathologically by infiltration of inflammatory cells into the vascular endothelium and marked intimal hyperplasia of smooth muscle cells (SMCs). These changes lead to a reduction in vascular lumen size, progressive blood flow compromise, and ultimately ischemic graft failure. Given the diffuse nature of TV involving epicardial and intramural arteries and veins of the transplanted heart, traditional therapies to treat areas of coronary artery stenosis, such as percutaneous coronary intervention with stents, are ineffective, favoring re-transplantation as the only definitive treatment option [Mitchell and Libby, 2007; Boilson and McGregor, 2009]. The incidence of TV has been slowly declining in the current era, likely due to improvements in immunosuppressive medications; however, TV remains a leading cause of morbidity and mortality after transplantation [Stehlik et al., 2012]. Innate and adaptive immune responses, in addition to non-immune factors, have all been linked to the pathogenesis of TV [Caforio et al., 2004; Pinney and Mancini, 2004; Mitchell and Libby, 2007; Boilson et al., 2011; Stehlik et al., 2012]. There is growing evidence that TV is a chronic, delayed type hypersensitivity reaction directed against donor ECs and SMCs fueled by cytokine and chemokine mediated inflammation and ongoing cellular recruitment [Valantine, 2004; Mitchell and Libby, 2007].

STEM CELLS AND TRANSPLANT VASCULOPATHY

Endothelial progenitor cells (EPCs) are able to repopulate most cell types in the human heart. In 2002, two groups independently discovered between 0.04% and 18% of cardiomyocytes in the transplanted human heart were of recipient origin [Laflamme et al., 2002; Quaini et al., 2002]. Both reports studied "chimerism in the organ" in male patients who underwent gender mismatched heart transplantation from female donors. This allowed detection of cells containing the Y-chromosome to serve as an extracardiac lineage marker [Laflamme et al., 2002]. Quaini et al. [2002] concluded that: (1) chimerism in the organ was present in all transplanted hearts, (2) cells containing the Y-chromosome were found in cardiac muscle (18%), coronary arterioles (20%), and capillaries (14%), (3) significant cellular differentiation occurred from primitive stem cells and committed (precursor) progenitor cells, however, the location of precursor cells was not identified, and (4) migration of recipient progenitor cells into the allograft and complete cell differentiation was a rapid (4 day) process. Using a similar gender mismatched human transplant model, Minami et al. [2005] reported chimerism specifically in ECs (24%), SMCs (3%), and peripheral nerve (11%) cells. Interestingly, ECs chimerism was more common (23–36%) in the coronary microvasculature ($\leq$100 μm diameter), however, it did not differ in frequency between arteries and veins. Simpler et al. [2003] assessed peripheral blood from cardiac transplant patients and found that EPCs, but not circulating ECs, were decreased in subjects with known TV compared to subjects without TV. Additionally, they demonstrated a high level of endothelial chimerism in diseased (i.e., TV) versus non-diseased endothelial vascular segments, suggesting infiltration of recipient EPCs at sites of more severe TV [Simper et al., 2003]. Sieveking et al. [2008] functionally sub-divided human EPCs into "early" and "late" outgrowth progenitor cells types. Early EPCs did not directly participate in angiogenesis; however, they contributed to angiogenesis in a paracrine fashion, as opposed to late EPCs, which were found to directly incorporate into vascular networks. These data suggest that different functional roles for EPCs exist, which may have vastly different therapeutic implications regarding choice of EPC sub-type and timing of cellular therapy for vascular reparative strategies in TV. There is, however, ongoing debate as to which cellular antigen markers "define" EPCs [Case et al., 2007; Sieveking et al., 2008]. Case et al. [2007] reported that the identification of EPCs by CD 34 and AC 133 antigens, along with surface receptor VEGFR-2, may actually identify a hematopoietic progenitor cell (HPC) population. HPCs do not differentiate into endothelium; however, they do contribute to angiogenesis and EC function via paracrine activity by producing of vascular growth factors, such as VEGF [Majka et al., 2001; Boilson and McGregor, 2009]. Additionally, HPCs have recently been the focus of developing strategies to induce long-term immune tolerance in solid organ transplantation by

hematopoietic chimerism [Joffre et al., 2008; Boilson and McGregor, 2009; Pasquet et al., 2011]. Specifically, Joffre et al. elegantly demonstrated induction of skin and cardiac allograft tolerance in a mouse model by manipulating recipient T lymphocytes and donor antigen presenting cells (APCs) and was able to prevent both acute and chronic forms of rejection, both of which are important in the development of TV [Mitchell and Libby, 2007; Joffre et al., 2008; Boilson and McGregor, 2009; Boilson et al., 2011]. Determining the biologic potential of cells is critical in understanding progenitor and stem cell involvement for cardiac neovascularization and vascular reparative strategies.

STEM CELLS AND TRANSPLANT VASCULAR REMODELING

Progressive TV leads to decreased arterial luminal diameter due to changes in the composition of the vascular wall [Mitchell and Libby, 2007]. Infiltration of inflammatory cells within the tunica intima and proliferation of SMCs leads to decreased vascular luminal diameter. However, luminal size is dynamic, with medial SMC death and remodeling via proteolytic enzymes balanced by adventitial remodeling and the direct effects of arterial vasomotor tone [Pethig et al., 1998; Hillebrands et al., 2003; Laflamme et al., 2006]. Interestingly, although medial SMCs are donor derived, intimal SMCs are predominantly recipient derived, and occur in different proportions in animal versus human models [Mitchell and Libby, 2007]. Glaser et al. investigated the contribution of recipient SMCs in human cardiac allografts and found that up to 16% of SMCs located in medium and small arteries were of definitive recipient origin. Phenotypic differences between intimal and medial SMCs also exist, suggesting different progenitor cell origin [Glaser et al., 2002; Hillebrands et al., 2003]. Several animal studies have attempted to determine if intimal SMCs were of BM or non-BM stem cell origin, finding a wide range (11–82%) of intimal SMCs were of BM origin [Han et al., 2001; Li et al., 2001; Saiura et al., 2001; Sata et al., 2002]. Hillebrands et al. [2005] suggest that mesenchymal progenitor cells found in the adventitia may contribute to intimal SMC proliferation due to recruitment through the vascular wall and into the sub-endothelial space, or from direct release into the blood as circulating progenitor cells. Alternatively, intimal SMCs may arise from adult EPCs functioning as SMC progenitor cells [Yamashita et al., 2000]. Blood borne origins of intimal SMCs have been reported when outgrowth from in-vitro human mononuclear blood cultures demonstrated SMC phenotypic characteristics such as SMA, myosin, and calponin [Hillebrands et al., 2003; Simper et al., 2003]. Given the differences in reported intimal SMC precursor cell origins, it is likely that intimal SMCs have considerable precursor cell plasticity. Hillebrands et al. [2003] found that SMC precursor cell origin depends on the chronicity of vasculopathy, degree of vascular damage and inflammation, and the location of vascular injury. In fact, BM-derived intimal SMCs were identified only in arteries with severe vascular damage, but not in arteries with only minimal damage. Additionally, the severity of vascular damage was found to be important for the differentiation of BM stem cells in general and specifically into intimal SMC phenotypes [Han et al., 2001; Hillebrands et al., 2003]. In summary, intimal SMCs likely arise from multiple precursor cell types and locations based on recruitment signals driven by specific vascular characteristics. Therefore, development of therapeutic interventions for patients with TV should focus on identifying intimal SMC precursor cell types, recruitment pathways and factors leading to precursor cell differentiation.

IMMUNOSUPPRESSIVE THERAPY EFFECT AND STEM CELLS

Improved immunosuppressive therapies with calcineurin inhibitor, anti-proliferative agents, and steroids have decreased the cumulative incidence of TV over time [Stehlik et al., 2012]. Recently, proliferation signal inhibitors such as sirolimus have been shown to decrease TV-associated intimal proliferation [Raichlin et al., 2007; Topilsky et al., 2012]. In addition to modulation of specific cytokine and T-cell functions, sirolimus has potent inhibitory effects on both endothelial and smooth muscle progenitor cells [Butzal et al., 2004; Fukuda et al., 2005]. Sathya et al. assessed EPCs in peripheral blood samples from transplant patients stratified by rejection episodes and sirolimus use. They found higher EPC colony forming units (CFUs) in patients with an allograft rejection history; however, in patients taking sirolimus, there was reduced EPC CFU's irrespective of rejection history [Sathya et al., 2010]. In animal models, cylcosporine, a calcineurin inhibitor, reduced CFUs of endothelial and smooth muscle progenitor cellss, however, there was recovery of progenitor CFUs found at the time of study completion despite therapeutic levels of immunosuppression [Davies et al., 2005]. These findings suggest an adaptive response of progenitor cells to cyclosporine therapy and warrant further investigation. Defining the association between EPCs and immunosuppressive therapies may be important in determining if specific immunosuppressive medications have differential effects on the vascular reparative qualities of EPCs in patients with TV.

Administration of statin medication after cardiac transplantation has been associated with less frequent cardiac rejection, a lower incidence of TV, and decreased natural killer cell cytotoxicity [Kobashigawa et al., 1995]. In a murine model, statin treatment was associated with accelerated re-endothelialization after induced vascular damage, and decreased intimal thickening as a consequence of statin-induced mobilization of BM-derived EPCs [Llevadot et al., 2001; Walter et al., 2002]. Kusuyama et al. [2006] incubated human peripheral blood with statin medication and found that among BM-derived vascular progenitor cells, statin medication promoted EPC differentiation and inhibited differentiation and mobilization of SMC progenitor cells. Yin et al. [2007] found that statin mediated inhibition of monocyte chemoattractant protein-1, RANTES chemokine, and chemokine receptors (CCR2 and CCR5), was associated with decreased immune cell vascular recruitment and less TV development. In summary, statins appear to decrease TV in three broad but distinct ways: (1) statins promote EPC differentiation from BM progenitor cells, (2) decrease MHC-II-mediated T-cell vascular insults thus mitigating acute and chronic forms of rejection, and (3) decrease cholesterol oxidation-mediated vascular inflammation [Kobashigawa, 2004]. Further research into the mechanisms of immunosuppressive medications, including statins, and effects of these medications on vascular progenitor cells will be critical in developing therapies to minimize or prevent TV.

CAN STEM CELLS PREDICT TV RISK?

Chronic endothelial dysfunction is a hallmark of TV, therefore, measurement of endothelial dysfunction may be useful in predicting outcomes in patients with TV. EPCs have a role in vascular homeostasis

and contribute to endothelial repair; therefore, measuring the number of EPCs may be a surrogate biologic marker of vascular function and overall cardiovascular risk [Walter et al., 2002; Sathya et al., 2010]. Hill et al. correlated EPC CFUs in peripheral blood from humans with cardiovascular risk, as measured by the Framingham risk score, and EC functions measured by brachial artery ultrasound. They found significant reductions in EPCs in patients chronically exposed to hypertension, hyperlipidemia and diabetes. Additionally, the Framingham risk score was strongly inversely correlated with EPC counts. Finally, in subjects with high cardiovascular risk, EPCs were found to become prematurely senescent, coinciding with observations in animal studies that stem cell exhaustion limits longevity [Geiger and Van Zant, 2002; Tyner et al., 2002]. It is necessary to develop a test utilizing EPCs as a quantitative biological marker of "vascular health" or more specifically, TV severity. This could be useful clinically to refine TV assessment and direct management strategies, however may be most important as a means to objectively assess the efficacy of future TV disease modifying therapies.

STEM CELLS AFTER CARDIAC TRANSPLANTATION: WHERE TO GO FROM HERE?

Progression of TV occurs due to a mismatch between two biologic processes: endogenous vascular repair, likely driven by EPCs, and progressive innate and adaptive immune-mediated vascular injury. Endothelial regenerative strategies using genetic manipulation of EPCs has been performed in animal models, resulting in accelerated endothelial regeneration, increased incorporation of EPCs into vascular endothelium and attenuation of TV [Feng et al., 2008, 2009; Lim et al., 2013]. Hematopoietic chimerism is another attractive therapeutic option to induce allograft tolerance and therefore eliminate or minimize TV related to chronic rejection. This has been successfully accomplished in animal models undergoing heart transplantation and in human models undergoing renal transplantation [Pasquet et al., 2011, 2013; Leventhal et al., 2012, 2013; Yamada et al., 2012; Kawai and Sachs, 2013]. Advances in regenerative tissue engineering using autologous stem or progenitor cells hold great potential to create a bioartifical heart [Taylor, 2009]. Recent developments have successfully decellularized a murine heart and recellularized the cardiac scaffolding with pluripotent stem cells to give rise to a beating murine heart [Ott et al., 2008; Taylor, 2009; Badylak et al., 2011; Lim et al., 2013]. It is tantalizing to consider a day when stem cells are used to create a personalized organ, thereby addressing issues from donor organ shortages, immunosuppression side effects, and TV. Given the inherent biological complexity of TV, stem cell therapies will need to be combined with immunomodulatory-based therapies to comprehensively treat TV. However, utilization of autologous stem cells to create new organs holds the greatest promise to fundamentally change the future of cardiac transplantation.

STEM CELLS AND MECHANICAL CIRCULATORY SUPPORT FOR HEART FAILURE

End-stage HF is characterized by progressive cardiomyocyte hypertrophy, cellular apoptosis, myocardial fibrosis, and eccentric ventricular remodeling, leading to severe chamber dilation [Gajarsa and Kloner, 2011]. These macro level changes are a result of pathological changes occurring at the microscopic level with dysregulation of calcium metabolism and altered gene expression of myocyte contractile proteins. Unfortunately, chronic pathologic remodeling is usually terminal, with heart transplantation being the only definitive treatment.

Donor hearts for transplant are scarce: 6–10% of the population over 65 years of age in the US suffers from HF, however, only 2,300 donor hearts are available annually [Westaby, 2008]. The number of donor hearts has remained unchanged over the last decades despite an increasing demand and longer transplant waiting times [Stehlik et al., 2012]. Therefore, long-term mechanical circulatory support with LVAD is being increasingly utilized as a bridge to transplantation strategy until a suitable donor organ becomes available. Additionally, patients who are ineligible for transplantation due to age or co-morbidities can receive a LVAD for destination therapy indications, which currently represents the fastest growing patient group receiving long-term mechanical circulatory support [Kirklin et al., 2013]. Regardless of indication, LVADs have been shown to dramatically improve survival and HF functional class in patients who historically would have had few options other than palliative care [Slaughter et al., 2009]. Mechanistically, LVADs improve circulation by mechanically unloading the failing LV, which decreases LV strain and shrinks ventricular chamber size, a process termed "reverse remodeling." However, LVADs have also been shown to induce cellular, molecular, and genetic changes in cardiomyocytes, thus providing hope for myocardial recovery [Birks, 2010; Mann and Burkhoff, 2012]. Recent evidence suggests that reverse cardiac remodeling during LVAD support may be aided by an increase in recruitment or proliferation of regenerative cell types, including mast cells [Jahanyar et al., 2008], cardiomyocytes, and stem cells [Wohlschlaeger et al., 2012].

LVADs AND PATIENT STEM CELLS

Although cardiomyocytes are not typically believed to undergo cell division and proliferation in adults, LVAD support may promote reverse remodeling through the regeneration of cardiomyocytes. Failing cardiomyocytes replicate DNA without successfully completing mitosis, resulting in increased polyploid cardiomyocytes [Sandritter and Adler, 1976; Adler and Sandritter, 1980]. Interestingly, Wohlschlaeger et al. [2010] demonstrated that the number of polyploid cardiomyocytes decreased, while the number of diploid cardiomyocytes increased, in a group of end-stage HF patients with LVAD therapy; however, no changes were noted in patients who were not supported with LVAD therapy. Mechanical unloading by LVADs may trigger the release of cytokines and hormones which promote cardiomyocyte healing and allow for the successful completion of karyokinesis and thus a change from polyploid to diploid cardiomyocytes [Wohlschlaeger et al., 2005]. However, despite extensive efforts, direct karyokinesis from polyploidy to diploid cardiomyocytes has not been observed in this setting [Wohlschlaeger et al., 2005, 2012]. The increase in diploid cardiomyocytes during LVAD support may alternatively be explained by an increase in the cardiac progenitor and stem cell populations [Wohlschlaeger et al., 2012]. In particular, Wohlshlaeger

et al. [2012] observed an increase in cardiac c-Kit$^+$/MEF-1$^+$ stem cells as well as side population cells (SPCs), which are ATP-binding cassette transporters ABCG1 (multi-drug resistance gene product 1) and ABDG2 (breast cancer resistance protein) positive, in patients supported by LVADs. These SPCs and stem cells can differentiate into cardiomyocytes as well as other cell types, including ECs and SMCs, to further aid in tissue repair. The source of these stem and progenitor cells is still unclear: they may have been recruited to the myocardium or may have been the result of proliferation of resident progenitor cells upon mechanical unloading [Wohlschlaeger et al., 2012]. Although this finding is exciting, the population of these SPCs and stem cells in the mechanically unloaded myocardium is extremely limited [Wohlschlaeger et al., 2012], indicating the need to further increase the number of CSCs in patients with LVADs.

COMBINING STEM CELLS AND LVAD THERAPIES

As previously reviewed, stem cell transplantation is known to improve perfusion in ischemic heart tissue, although efficacy is limited by delivery efficiency, engraftment, and survival [Barbash et al., 2003; Murry et al., 2004; Freyman et al., 2006]. To improve efficacy, stem cells can be delivered concomitantly with LVAD support to promote myocardial recovery. For example, Anastasiadis et al. [2011] demonstrated that LVAD support increased the viability and survival of implanted stem cells by myocardial unloading, improved coronary circulation, and reduced inflammation. Additional work from Anastasiadis et al. [2012] reported that when human autologous BMCs consisting of EPCs (CD133$^+$), hematopoietic stem cells (CD34$^+$) and mesenchymal stem cells (CD105$^+$) were injected into a severely ischemic myocardium supported with a Jarvik 2000 LVAD, the LVEF improved from 15% preoperatively to 45% after 1 year follow-up. This report suggests a hybrid approach utilizing stem cells in the context of mechanical circulatory support may facilitate myocardial recovery. Stem cells have also been used in combination with other cell types to encourage cardiac repair in patients supported with LVADs [Fujita et al., 2011]. Skeletal myoblasts have proven capacity for cardiac myocyte regeneration [Menasche et al., 2001, 2008; Dib et al., 2005] and have been implanted in combination with autologous BMCs into four patients supported with LVADs. Decreased brain natriuretic peptide (BNP) levels and improved LVEF were observed in two of these patients, including successful LVAD explantation in one patient [Fujita et al., 2011]. Further work assessing the optimal stem cell type, timing and method of stem cell delivery, and optimal way to assess efficacy of stem cell therapy in patients supported with LVADs needs to be determined. Also intriguing is whether the type of LVAD (centrifugal vs. axial continuous flow) and degree of mechanical unloading will effect myocardial recovery, with or without concurrent stem cell therapy. Currently, two clinical trials are underway to evaluate the effects of stem cells therapy in patients supported with LVADs (clinical trial identifier: NCT01442129 and NCT00869024). BM-derived cells will be injected into the heart during LVAD implantation. Outcomes that will be measured include the safety of stem cell therapy in this population and efficacy of therapy for myocardial functional recovery. No clinical data from these trials have been reported so far. The results of these cutting edge trials will provide mechanistic insight into the process of reverse remodeling and information regarding the durability of a hybrid approaches using stem cell therapy and mechanical circulatory support.

CONCLUSION

Cardiac-based stem cell therapies have limited capability as a stand-alone therapy to impact the current therapeutic needs in advanced HF. However, the utility of stem cells to modify and repair damaged myocardium is evolving. The implantation of stem cells in patients with heart transplantation or supported with an LVAD have shown encouraging results in reversing cardiac remodeling and improving myocardial function.

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