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Forum Review Article

Heart Failure Management: The Present and the Future

Mohammad N. Jameel and Jianyi Zhang

Abstract

Clinical heart failure has been defined for a long time as a clinical *syndrome* with symptoms and signs including shortness of breath, cyanosis, ascites, and edema. However, in recent years, with the thought of promoting early diagnosis and heart-failure prevention, the concept of heart failure has often been defined simply as a subject with severe LV dysfunction and a dilated left ventricle, or by some, defined by evidence of increased circulating levels of molecular markers of cardiac dysfunction, such as ANP and BNP. Heart failure has been considered an irreversible clinical end point. Current medical management for heart failure only relieves symptoms, slows deterioration, and prolongs life modestly. However, in the recent years, rejuvenation of the failing myocardium began to seem possible as the accumulating preclinical studies demonstrated that rejuvenating the myocardium at the molecular and cellular level can be achieved by gene therapy or stem cell transplantation. Here, we review selected novel modalities that have been shown in preclinical studies to exert beneficial effects in animal models of severe LV dysfunction and seem to have the potential to make an impact in the clinical practice of heart-failure management. *Antioxid. Redox Signal.* 11, 1989–2010.

Introduction

Clinical heart failure is traditionally defined as a syndrome with specific symptoms and signs, including shortness of breath, cyanosis, ascites, and edema. It can result from abnormalities of the pericardium, myocardium, endocardium, or the great vessels, but the majority of cases are associated with myocardial dysfunction. Coronary artery disease, hypertension, and dilated cardiomyopathy are the most important causes in the Western world.

Heart failure is a major public health issue in the United States and around the world. About 550,000 new cases occur each year in the United States, and the estimated cost in 2007 was >33 billion dollars (138). The lifetime risk of heart failure in the US is 20% (99). Although data from developing countries are scant, it is estimated that 23 million people have heart failure around the world (107).

We initially briefly review the current medical management of clinical heart failure, and then discuss novel drug therapies, followed by in-depth discussion of potential gene and stem cell therapies in heart failure.

Current Management of Heart Failure

The management of clinical heart failure begins with prevention, which is aimed at treating the modifiable risk factors. Once a patient develops structural heart disease and symptoms of heart failure, the management includes the following therapeutic strategies.

Diuretics

Diuretics interfere with sodium retention in heart failure and have been shown to improve symptoms in patients with decompensated clinical heart failure but do not affect outcome.

Inhibitors of the renin–angiotensin–aldosterone Pathway

Although many factors are involved in the acceleration of left ventricular remodeling, activation of endogenous neurohormonal mechanisms plays an important role in cardiac remodeling, and thus their modulation might be beneficial in

Department of Cardiology, University of Minnesota, Minneapolis, Minnesota.

patients with heart failure. Angiotensin-converting enzyme inhibitors (ACEIs) block the production of angiotensin II from angiotensin I; angiotensin-receptor blockers (ARBs) block angiotensin II at the receptor level; and aldosterone antagonists block at the aldosterone-receptor level. ACEIs have been evaluated in >7,000 patients (mostly reduced EF) in >30 placebo-controlled trials (47). These studies show that ACEIs improve symptoms, reduce hospitalization, and decrease mortality in patients with heart failure. ARBs can be used in patients who are intolerant to ACEIs, with similar benefit (52, 106). Aldosterone antagonists were shown to reduce further the risk of hospitalization and death in patients with NYHA class III and IV heart failure in two different clinical trials (129, 130).

Beta blockers

Beta blockers inhibit the adverse effects of activation of the sympathetic nervous system in patients with heart failure. They have been evaluated in >20,000 patients in >20 placebo-controlled trials that showed that beta blockers improve symptoms, reduce hospitalizations, and decrease mortality in patients with heart failure (30, 32).

Isosorbide dinitrate and hydralazine

A combination of isosorbide dinitrate and hydralazine, in addition to standard therapy, has been shown to improve survival in black patients with heart failure (161).

Digitalis

Digitalis glycosides inhibit the Na-K ATPase in cardiac cells to increase contractility (4), but also in vagal afferent fibers and kidneys, which helps modulate the neurohormonal imbalance in heart failure (49, 162). Placebo-controlled trials showed that treatment with digoxin improves symptoms in heart failure but has no effect on mortality (1, 48). However, it has a narrow therapeutic window and should be used with caution.

Cardiac resynchronization therapy

Cardiac resynchronization therapy (CRT), when added to optimal medical treatment in patients with persistent heart failure, resulted in improvement of quality of life and survival (2).

Left ventricular assist devices and cardiac transplantation

In end-stage heart failure patients, left ventricular assist device (LVAD) implantation and ultimately cardiac transplantation might be the last resorts available. Cardiac transplantation is limited by the number of donor hearts (111).

Novel Pharmacologic Interventions

Nitrite treatment

The anion nitrite (NO_2^-) was previously considered physiologically inert, but recent studies showed that nitrite acts as a biochemical reservoir of nitrous oxide (NO). It is converted into NO by hemoglobin, myoglobin, and other metal-containing enzymes, and this conversion is enhanced under ischemic conditions.

Nitrite therapy before or during ischemia/reperfusion (I/R) injury exerts a beneficial effect on the heart (42, 50, 171), as summarized in Table 1. Nitrite treatment before coronary artery occlusion and reperfusion in an isolated rat heart with the Langendorff perfusion model resulted in reduction in scar size and improved ventricular function (171). In an *in vivo* murine myocardial ischemia/reperfusion model, nitrite treatment during ischemia reduced the infarct size by 67% (42). The effective dose of nitrite in this study was ~48 nmol, and the blood concentration was ~10 μM for optimal effect (42). Recently, a prospective placebo-controlled study in a canine I/R model examined the effects of nitrite treatment and concluded that it increased microvascular perfusion, reduced apoptosis, and improved contractile function (51). The exact mechanism of cytoprotection as a result of nitrite treatment is still unclear, but it seems to be related to nitrite-dependent NO production. This is shown by the abolishment of beneficial effect in these studies after treatment with NO scavengers such as PTIO [2-(4-carboxyphenyl)-4,4,5,5-tetramethylimidazole-1-oxyl 3-oxide] (42, 171). This effect of nitrite is also independent of eNOS and heme-oxygenase-1, as pretreatment with inhibitors of eNOS and heme-oxygenase-1 did not affect the beneficial effects (42). Similarly, nitrite resulted in an improved cardiac function in eNOS KO and heme-oxygenase KO mice (42). NO and the NO-modified proteins and lipids then exert their downstream beneficial effects through the following mechanisms (38):

1. Inactivating proteins involved in apoptosis (for example, S-nitrosation of caspases).
2. Protecting against cell death by opening mitochondrial KATP channels, which prevents mitochondrial cytochrome *c* release, and the opening of the mitochondrial permeability transition (MPT) pore.
3. Decreasing production of reactive oxygen species (ROS) by inhibition of complex I or by inhibition of complex IV, which slows mitochondrial respiration.
4. Antiinflammatory effects.

Hydrogen sulfide (H_2S)

H_2S is a well-known gas, often used to describe the smell of rotten eggs. It has recently been shown to be produced endogenously and is present in 10- to 100-μM concentration in the circulation. It is an important gaseous signaling molecule that exerts several physiologic actions in the cardiovascular system, including vasodilation, antioxidant effects, inhibition of apoptosis, and transient and reversible inhibition of mitochondrial respiration. Because of these effects, H_2S therapy has been studied in animal models of cardiovascular diseases (Table 2). The most clinically relevant animal study administered Na_2S (an H_2S donor) at the time of reperfusion in a mous model of ischemia/reperfusion that resulted in a decrease in infarct size and improvement in LV function (43). This was related to decrease in myocardial inflammation, reduction in apoptosis, and preservation of mitochondrial structure and function (43). Larger-animal model preclinical studies are required before envisioning clinical trials for this exciting therapy.

Gene Therapy in Heart Failure

Our understanding of the molecular mechanisms involved in the pathogenesis of heart failure has increased tremen-

TABLE 1. NITRITE TREATMENT IN ANIMAL MODELS OF ISCHEMIA/REPERFUSION

Author	*Animal model*	*Study protocol*	*Therapy*	*Placebo*	*Outcome*	*Year*	*Reference*
Webb A	Isolated perfused rat heart	60-min ischemia and 30-min reperfusion	0.025–2.5 μM constant infusion for 15 min before ischemia onset	Saline	Reduction in infarct size and improvement in LV function	2004	171
Duranski MR	Mice	30-min LM occlusion and 24-h reperfusion	2.4–1,920 nmol intraventricular infusion midway through ischemic time	Nitrate or saline	Reduction in infarct size and improvement in LV function	2005	42
Baker JE	Rat	30-min regional ischemia and 3-h reperfusion	0.04–10 mg/kg IV bolus 15 min before ischemia onset	Nitrate	Reduction in infarct size and improvement in LV function	2007	11
Gonzalez FM	Canine	2-h LAD occlusion and 6-h reperfusion	0.20 μmol/min/kg for 20 min followed by 0.17 μmol/min/kg for 40 min OR 0.20 μmol/min/kg for 5 min, ending at reperfusion	Saline	Reduction in infarct size and improvement in LV function	2008	51

dously over the past decade. This allows us to target tissue of organ treatment at key molecular entities within these complex pathways in hopes of restoring normal cellular function and consequently better organ function. This section initially discusses the vehicles for gene-therapy and gene-delivery techniques and then reviews the most significant molecular targets for gene therapy in heart failure.

Vehicles for gene therapy

Successful gene therapy requires efficient myocardial specific transduction and long-term transgene expression. Only viral vectors have been shown to meet these requirements, and of these, adenoviruses (AdVs) and adeno-associated viruses (AAVs) are the most commonly used. The advantages of AdVs are that they have excellent tissue-specific myocardial transduction, large transgene cloning capacity (7–8 kb), can be easily manipulated, and can be produced in high titers (172). However, *in vivo* delivery leads to an inflammatory response that causes a transient transgene expression, and furthermore, a repeated delivery results in a secondary immune response (28). These side effects can be minimized by using the newer "gutted" AdVs, which lack the immunogenic epitopes. The advantages of AAVs are that they are less immunogenic and have excellent long-term stable transgene expression (172). Moreover, some serotypes display tropism toward cardiac tissue (124). However, they can carry only small amounts of genetic material (4–5 kb), and some have inherent antibodies in humans, limiting their use (59).

Gene-delivery techniques

Gene delivery has been performed in animal models through the intravenous, intracoronary, and direct intramyocardial routes. An intravenous approach may be ideal but may prove to be impossible in humans, as the large blood volume dilutes the effective concentration of viral vectors reaching the myocardium. In mice and rats, large doses of viral vectors have been used, and the use of these or even higher doses are required for effective transduction in humans, which may not be safe. Intracoronary delivery is clinically applicable; however, this approach is also generally inefficient unless certain adjuvant measures are taken to enhance transduction. In large-animal models, blocking venous outflow, retrograde infusion, and closed-loop systems have been used for these purposes (61, 79, 135). Direct intramyocardial injection results in good transduction and can be used at the time of cardiovascular surgery (158).

Molecular targets for gene therapy in heart failure

A summary of key molecular targets for gene therapy for heart failure are summarized in Table 3 and illustrated in Fig. 1.

Improving perfusion. Ischemic heart disease is the most important cause of congestive heart failure, and thus, angiogenic gene therapies aimed at improving perfusion could be extremely beneficial in this setting. An adenovector expressing vascular endothelial growth factor (VEGF121 cDNA) resulted in collateral vessel formation and improved perfusion and function in a chronic ischemic swine model (102). An adenoviral administration of VEGF also caused a restoration of cardiac function in a pacing-induced heart-failure model in

TABLE 2. HYDROGEN SULFIDE TREATMENT IN ANIMAL MODELS OF ISCHEMIA/REPERFUSION

Author	Animal model	Study protocol	Therapy	Placebo	Outcome	Mechanism	Reference
Bian JS	Isolated perfused rat hearts	Low-flow ischemia for 30 min and 10-min reperfusion	3 cycles of 3 min of perfusion with NaHS 100 μM ($\approx$33 μM H_2S) separated by 5 min of superperfusion by normal Krebs solution preceding ischemia	No treatment	Reduction of IR-induced arrhythmias, increased cell viability	Sarcolemmal K-ATP channel, protein kinase C	(18)
Hu Y	Isolated perfused rat hearts	Low-flow ischemia for 30 min and 10-min reperfusion	3 cycles of 3 min of perfusion with NaHS, 100 μM, separated by 5 min of superperfusion with normal Krebs solution preceding ischemia	No treatment	Decreased infarct size and improved contractile function	Sarcolemmal K-ATP channel, protein kinase C, ERK 1/2, PI3K/Akt	(69)
Sivarajah A	Rat	LAD occlusion for 25 min and 2-h reperfusion	NaHS, 3-mg/kg IV bolus 15 min before ischemia	Saline	Decrease in infarct size	Antiapoptotic and antiinflammatory	(152)
Elrod JW	Mouse	30-min ischemia and 24-h reperfusion	NaHS at 50-μg/kg at the time of reperfusion	Saline	Decreased infarct size and improved LV function	Inhibition of myocardial inflammation and preservation of mitochondrial function	(43)

swine (91). Several clinical trials of angiogenic therapies have been performed in CAD patients. The phase II Kuopio angiogenesis trial (KAT) showed improvement in myocardial perfusion at 6 months after intracoronary administration of Ad-VEGF165 in patients undergoing angioplasty but no difference in restenosis rate (63). Intracoronary fibroblast growth factor (AdFGF4) delivery in patients with stable CCS II/III angina resulted in improved exercise tolerance at 4 weeks in the phase I/II AGENT 1 trial (53) and significantly decreased ischemic defects in the phase II AGENT 2 trial (54). However, the larger multinational multicenter placebo-controlled phase III AGENT 4 trial failed to show a significant difference in exercise tolerance at 12 weeks. No clinical trials of angiogenic therapies have been performed in heart-failure patients (13).

Targeting proteins involved in cardiomyocyte calcium handling. The handling of calcium during excitation contraction (EC) coupling is abnormal in failing hearts. Several molecular targets in this pathway have been used in gene therapy.

SR Ca^{2+} ATPase. The SR Ca^{2+} ATPase in the myocytes is known as SERCA2a, and its activity is reduced in heart failure, resulting in decreased calcium uptake and impaired relaxation (60). SERCA2 activity is controlled by phospholamban (PLN); dephosphorylated PLN inhibits SERCA2a, whereas phosphorylated PLN reduces this inhibition (84). SERCA2a gene therapy would aim to increase SERCA2a activity, resulting in quicker calcium uptake and thus improved diastolic relaxation; it also would increase contractile reserve because of higher SR calcium concentration. SERCA2a gene therapy in isolated cardiomyocytes from failing human hearts restored their contractile function (36). A number of small-animal studies have shown the beneficial effects of SERCA2a gene therapy (37, 113). Rats treated with intracoronary Adv-SERCA2a after transaortic constriction (TAC) showed improved systolic and diastolic function along with improved survival at 28 days (37, 113). Moreover, transgenic SERCA2a mice had improved function after TAC (70), whereas SERCA2a heterozygous KO mice developed LV dilatation faster (147). These beneficial effects have been confirmed in large-animal models of heart failure, in which AAV1-SERCA2a was given though an antegrade coronary artery infusion, resulting in improved contractile function at 2 months (78). However, some reports exist of increased mortality secondary to arrhythmias after SERCA2a treatment in rats with MI (26).

Currently, two phase I trials are under way, testing the effects of SERCA-2a gene delivery in heart-failure patients. CUPID (Calcium Upregulation by Percutaneous Administration of Gene Therapy in Cardiac Disease) first aims to identify the safe and efficient dose of AAV1-SERCA2a delivery by antegrade epicardial coronary infusion in patients with ischemic and nonischemic cardiomyopathy and then to test the beneficial effects in a small randomized placebo-controlled study (58). All patients will receive intracardiac defibrillators because of the prevoiously mentioned risk of arrythmias (58). The other trial is assessing the safety and biologic effects of the delivery of AAV6-SERCA2a in patients undergoing LVAD placement.

Phospholamban. Mutant forms of PLN, such as phosphomimetic mutation at serine 16 (S16E), are associated with increased SERCA2a activity (29). AAV-PLN-S16E delivery

Table 3. Molecular Targets for Gene Therapy in Heart Failure

Mechanism	*Molecular target*
Improve perfusion	VEGF
	FGF
	HIF1α
Calcium handling	SERCA
	Phospholamban
	Protein phosphatase and inhibitor protein
	Parvalbumin
	S100A1
B-Adrenergic-receptor pathway	β-2 receptor
	GRK2
	Adenylate cyclase
Prevention of fibrosis and adverse remodeling	TGF-β
	HGF
	MCP1
	Kallikrein
Antioxidant	HO-1
	EcSOD
	CuZnSOD
	MnSOD
	Catalase
Antiapoptotic	Bcl-2
	p35
Cell-cycle activation	Cyclin A_2

resulted in improved cardiac function in hamsters with cardiomyopathy (67) and in postmyocardial heart-failure rats (71). This was confirmed in a large-animal model of cardiomyopathy in sheep after delivery of AdV-PLN-S16E (79). However, the enthusiasm generated from these studies is hampered by discovery of a mutated and deleted PLN gene in patients with inherited cardiomyopathies (57). These cardiomyopathies have also been created in mouse models (56, 145). These observations warrant more studies before a clinical application can be sought.

Protein phosphatase (PP) 1 and inhibitor protein (I) 1-2. PLN action is secondarily controlled by PP1, which is further modulated by phosphatase inhibitors I1 and I2. β-Adrenergic–receptor stimulation leads to increased phosphatase inhibitors I1 through protein kinase A (PKA), causing decreased PP1, ultimately modifying PLN activity and increasing SERCA2a activity (22). Intracoronary AdV-I-1 delivery to rats after TAC resulted in improved cardiac function (126), whereas AdV-I-2 resulted in improved function in cardiomyopathic hamsters (175). Large-animal studies are clearly needed before human application.

Parvalbumin. Gene therapy with parvalbumin, an EF-hand Ca^{2+} sequestering protein, can potentially provide an energy-independent removal of cystosolic calcium. AdV-parvalbumin delivery leads to an increased rate of calcium removal and an improved rate of relaxation in hypothyroid rat hearts (159). However, AdV-parvalbumin delivery to cardiomyocytes from dogs after TAC resulted in improved relaxation kinetics but depressed sarcomere shortening at higher parvalbumin concentrations (66). This was probably due to inadvertant calcium removal during systole. Further studies will clarify its role in gene therapy for heart failure.

S100A1. S100A1 belongs to the family of S100 proteins, which is the largest EF-hand Ca^{2+}–sequestering protein subfamily (114). S100A1 regulates both SERCA2a and ryanodine receptor (RyR), thus improving cardiomyocyte EC coupling

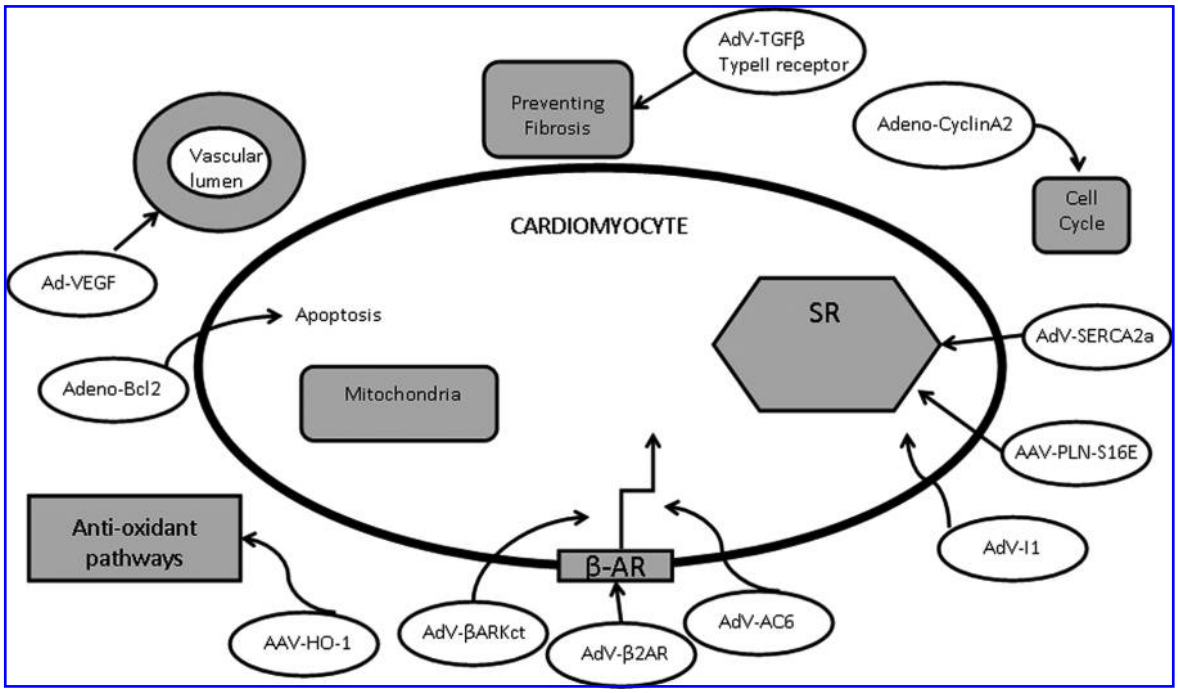

FIG. 1. Targets for gene therapy in heart failure. The transgenes have been used to improve perfusion, modulate calcium handling at the level of sarcoplasmic reticulum, manipulate β2 Adrenergic receptor signaling, prevent fibrosis, target apoptosis, target anti-oxidant pathways and target cell cycling.

and limiting arrhythmias through its stabilization of RyR and decrease in diastolic calcium leak (170). AAV6-S100A1 intracoronary delivery 10 weeks after MI in rat hearts resulted in improved cardiac function at 2 months (132). Intracellular calcium transients within a single cardiomyocyte also were restored (132). Preclinical studies with this particularly interesting molecular target are awaited.

Targeting the β-adrenergic system. β-Adrenergic blockers have been shown to be beneficial in heart-failure patients. Numerous basic science studies have shown that molecular targeting of various proteins within the β-adrenergic pathway may be beneficial in animal models of heart failure.

β_2-Adrenergic receptor overexpression. Overexpression of β_2-AR in mouse hearts results in improved systolic and diastolic function; however, with extremely high levels, mice develop fibrotic cardiomyopathy and heart failure after 40 weeks (94, 110). β_1-AR signaling leads to cell death and apoptosis, but β_2 signaling leads to cell-survival signaling and protects against apoptosis (31). These data led to the use of β_2-AR gene delivery in animal models of heart failure. AdV β2-AR intracoronary delivery in rabbits resulted in enhanced cardiac function (149). Further studies will determine its long-term effects and future clinical use.

Inhibition of GRK-2. Desensitization is the process by which kinases dampen the interaction between activated β receptors and their G proteins. Homologous desensitization (agonist dependent) is mediated by G protein–coupled receptor kinases (GRKs). GRK-2 is upregulated in human heart failure and is responsible for the desensitization. βARKct is a peptide within the carboxy terminus of GRK2 that has been used to inhibit GRK-2–mediated β-AR desensitization. βARKct gene transfer to isolated failing human cardiomyocytes improved their contractile function. Intracoronary delivery of AdV- βARKct resulted in improved function in post-MI rabbits. This exciting molecular target warrants further investigation.

Adenylate cyclase type 6. β-AR stimulation activates adenylate cyclase (AC) through G protein activation and leads to production of cAMP. AC then activates protein kinase to exert its downstream effects. AC5 and AC6 are the predominant cardiac isoforms (155), and, of these, cardiac AC6 overexpression leads to increased LV function and increased cAMP levels during β-AR stimulation while basal levels are normal (46). Intracoronary AdV-AC6 gene delivery in pigs with pacing-induced heart failure resulted in improved LV contractility (86). Further preclinical studies are awaited before clinical trials.

Preventing fibrosis. Myocardial infarction results in fibrosis and ultimate heart failure. Transforming growth factor (TGF-β) is involved in this process, and its inhibition may help prevent adverse remodeling. It has been shown that cardiac fibrosis is associated with recruitment of fibroblasts originating from endothelial cells, suggesting an endothelial–mesenchymal transition (EndMT) similar to events that occur during formation of an atrioventricular cushion in the embryonic heart (179). TGF-β induced the EndMT, whereas bone morphogenic protein 7 (BMP-7), a known antagonist of the TGF-β pathway, preserved the endothelial phenotype (179). Systemic administration of recombinant human BMP-7 significantly inhibited the EndMT and consequently decreased fibrosis and hypertrophy in a mouse model of pressure-overload hypertrophy (179). Adenoviral delivery of TGF-β type II receptor, which inhibits TGF-β in a post-MI mouse model, resulted in infarct contraction, reduction in fibrosis, and improvement in function (121). Postinfarction hepatocyte growth factor (HGF) administration *via* an adenoviral vector in mice resulted in improved LV remodeling and dysfunction, which was attributed to a reduced infarct wall thinning and antifibrosis (93). Inhibition of monocyte chemoattractant protein (MCP-1) by gene delivery of mutant MCP1 in a post-MI mouse model resulted in attenuation of cardiac dysfunction (62).

Targeting antioxidant pathways. Heme oxygenase 1 is a well-described molecule that exerts protective effects against increased oxidative stress in cardiovascular diseases. AAV delivery of HO-1 in an MI rat model resulted in improved cardiac remodeling and cardiac function (98). Similarly, pre-injury delivery of recombinant AAV carrying extracellular superoxide dismutase (EcSOD) in a rat model of ischemia/reperfusion resulted in improved left ventricular function and increased survival (3).

Targeting apoptosis. Apoptotic myocardial cell death contributes to the pathogenesis of heart failure. Molecular targets within the signaling pathway of apoptosis have been pursued as therapeutic targets in gene-therapy studies in animal models to reduce the cell-death burden. Adeno-Bcl2 gene delivery in a rabbit ischemia/reperfusion model resulted in decreased apoptosis and improved ejection fraction at 6 weeks (23). In a pacing model of heart failure in rabbits, a potent inhibitor of caspase (p35) administration *via* an adenoviral vector resulted in improved cardiac function (87).

Targeting cell-cycle activation. Another exciting possibility with gene therapy is the induction of endogenous myocardial regeneration. Adeno-cyclin-A_2 delivery in a post-MI rat model resulted in activation of the cardiomyocyte cell cycle, resulting in increased border-zone myofilament density and improved myocardial function (174).

Stem Cell Treatment in Heart Failure

A summary of the key clinical trials of cell transplantation in acute myocardial infarction and heart failure is shown in Tables 4 and 5. A description of the preclinical studies and clinical trials for the various stem cell types is discussed here.

Skeletal myoblasts

Skeletal myoblasts were the first cell type to be used in clinical trials. These cells can be transplanted in an autologous fashion without immunosupression and have several advantages, including high proliferative potential that allows an initial biopsy to be easily expanded *in vitro*. They also are terminally differentiated, thus decreasing the chances of tumorogenesis, and are known to be resistant to ischemia, allowing them to survive in scar or periscar areas where minimal perfusion is found. However, major drawbacks exist to skeletal myoblast transplantation. It has been shown in

TABLE 4. CLINICAL TRIALS OF STEM CELLS FOR CHRONIC ISCHEMIC DISEASE

Author	*Cell type*	*Mode*	*Number treated*	*Randomized*	*Primary end point*	p *Value*	*Result*
Menasche *et al.* (108)	Skeletal myoblasts	Open-heart surgery	127	Yes	LVEF	0.95	Decreased LV volumes
Dib *et al.* (40)	Skeletal myoblasts	Open-heart surgery	30	No	Safety and feasibility	—	Improved EF
Poznan (150)	Skeletal myoblasts	Transcoronary venous	10	No	Safety and feasibility	—	Improved NYHA class and improved EF
Perin *et al.* (127)	BMMNC	Endomyocardial	14	No	Safety	—	Improved EF and reduced symptoms
Fuchs *et al.* (45)	BMMNC	Endomyocardial	10	No	Safety and feasibility	—	Improved angina score, no change in EF
Tse *et al.* (165)	BMMNC	Endomyocardial	8	No	Safety and feasibility	—	No change in EF Improved wall thickening
TOPCARE-CHD (9)	CPC/BMMNC	Intracoronary	75	Yes	LVEF	0.003	EF improved after 3 mo, more in BMC than in CPC.
Erbs *et al.* (44)	GCSF-mobilized CPC	Intracoronary	13	Yes	CFR and LVEF	<0.05	LVEF and CFR increased at 3 mo

BMMNC, bone marrow mononuclear cells; CPC, circulating progenitor cells; GCSF, granulocyte colony-stimulating factor; EF, ejection fraction; CFR, coronary flow reserve.

animal studies that skeletal myoblasts do not differentiate into cardiomyocytes and are not electromechanically coupled to each other or to the surrounding cardiomyocytes (41, 90, 117). Phase I trials have demonstrated the feasibility of autologous skeletal myoblast transplantation in ischemic heart disease patients (64, 109, 123, 150, 151, 153). Engraftment of myoblasts has been documented in pathologic specimens up to 18 months after transplantation (55, 123). Definite concerns regard the development of ventricular arrhythmias in patients after myoblast transplantation (109, 151). These have prompted the use of intracardiac defibrillators (ICDs) in protocols for skeletal myoblast transplantation. The MAGIC trial was the first multicenter, randomized, placebo-controlled, double-blind study investigating the use of skeletal myoblasts in ischemic heart disease patients, and it failed to show an improvement in left ventricular function in patients receiving skeletal myoblasts at the time of coronary bypass grafting compared with those receiving revascularization alone, although the high-dose cell group showed a significant decrease in LV volumes in the subgroup analysis (108). All patients in this study were required to receive an ICD because of the potential risk of life-threatening arrhythmias. Despite a higher number of arrhythmic events in the myoblast-treated patients, the 6-month rates of major cardiac adverse events and of ventricular arrhythmias did not differ significantly between the treatment and placebo groups (108). In summary, the modest benefit after high-dose myoblast transplantation is offset by the increased risk of ventricular arrhythmias that requires the implantation of ICDs and adds to the cost and the patient discomfort. Other cell types may be better suited for cardiac regenerative therapy in the future.

Bone marrow–derived stem cells

The ability of bone marrow–derived cells to differentiate into cardiomyocytes is debated in the literature. Bittner *et al.* (19) were the first to suggest that cardiac muscle cells may be derived from BM cells. Goodel *et al.* (72) showed that after transplantation of murine BM side-population (SP) cells (c-kit$^+$, Sca-1$^+$, CD34-/low), donor-derived cells with cardiomyocyte morphology, as well as smooth muscle and endothelial cells were found in the heart after LAD ligation. Orlic *et al.* (122) showed that transplantation of GFP$^+$ Lin-c-kit$^+$ cells [presumably containing both hematopoietic stem cells (HSCs) and mesenchymal stem cells] into the ventricular wall after LAD ligation resulted in improved function of the ventricle, and they detected a large number of GFP$^+$ cells with a cardiac phenotype in the myocardium.

In contrast to these findings, other laboratories using genetic techniques showed that lineage-negative, c-kit–positive cells did not differentiate into cardiomyocytes (12, 116). However, more recently, Anversa and colleagues (139) showed, by using similar genetic techniques, that c-kit$^+$ bone marrow cells can engraft in proximity to the infarcted myocardium and differentiate into cells of the cardiogenic lineage, forming functionally competent cardiomyocytes and vascular structures.

Bone marrow cells (BMCs) were the second cell type to be tested in the clinical setting. The Transplantation of Progenitor Cells and Regeneration Enhancement in Acute Myocardial Infarction (TOPCARE-AMI) trial revealed significant improvement in LV ejection fraction, as well as significantly

Table 5. Clinical Trials of Stem Cell Treatment in Acute Myocardial Infarction

Author and reference	*Cell type*	*Mean cell dose ($\times 10^6$)*	*Number treated*	*Days after MI*	*Randomized*	*Primary end point*	p *Value*	*Result*
Strauer *et al.* (156)	BMMNC $CD34^+$ $AC133^+$	28	10	8	No	Safety	—	EF unchanged, regional contractility increased
Assmus *et al.* TOPCARE-AMI (10)	1. BMMNC CD34, CD45 5.5×10^6 CD34, CD133 0.28×10^6 2. Blood-derived progenitor cells (endothelial characteristics)	16 213	29 30	4.9	No	Safety and feasibility	—	EF increased
Wollert *et al.* BOOST (173)	BMMNC $CD34^+$ 9.5×10^6	2.460	30	4.8	Yes	LVEF	0.0026	Short-term benefit at 6 mo, but no difference between groups at 18 mo
Janssens *et al.* (73)	BMMNC 2.8×10^6 $CD34^+$ 2.0×10^6 $CD133^+$ 0.2×10^6 $CD90^+$/$Thy\text{-}1^+$ 2.5×10^6 $CD105^+$/endoglin 7.0×10^6 $CD117^+$/c-kit 32×10^6 $CD73^+$	304 nucleated cells and 172 mononuclear cells	33	<1	Yes	LVEF	0.36	Decreased infarct size
Chen *et al.* (25)	MSCs	48,000–60,000	34	18	Yes	LVEF	0.01	EF improved 14% above controls
REPAIR-AMI (143)	BMMNC $CD34^+CD45^+$ 2.5×10^6 CD133, CD45 1.9×10^6 $CD34^+CD133^+$ $CD45^+$ 1.8×10^6	1.8	1.1	3–7	Yes	LVEF	0.01	Improved EF at 4 mo
ASTAMI (100)	BMMNC $CD34^+$ 0.7×10^6 $CD133^+1.7\times10^6$	68	50	4–8	Yes	LVEF	0.7	No benefit at 6 mo
MAGIC Cell 3 DES (76)	GCSF mobilized PBSC 9.3% $CD34^+$ 15.1% KDR^+ 2.2% $AC133^+$ 5.7% CD34/KDR	1,400 leukocytes	25 16 (old MI)	4	Yes	LVEF	<0.05	EF improved at 6 mo in AMI; no improvement in old MI patients
STEMI (136)	GCSF-mobilized stem cells ($CD34^+$, $CD34^-CXCR4^+$)	None	39	<2	Yes	Systolic wall thickening	1.0	No benefit at 6 mo from GCSF treatment alone

BMMNCs, Bone marrow mononuclear cells; MSCs, mesenchmal stem cells; PBSC, peripheral blood stem cell; GCSF, granulocyte colony-stimulating factor; EF, ejection fraction.
Note: All are autologous cells delivered intracoronarily. BMMNCs are a mixed population of cells containing hematopoietic, mesenchymal, and other progenitor cells.

enhanced myocardial viability and regional wall motion in the infarct area after transplantation of BMMNCs or blood-derived progenitor cells (10, 142). The Bone Marrow Transfer to Enhance ST-elevation Infarct Regeneration (BOOST) study (173) also showed an increase in LV ejection fraction at 6 months with cell transplantation, but surprisingly, no difference was found between the treated and placebo groups at 18 months. The Reinfusion of Enriched Progenitor Cells and Infarct Remodeling in Acute Myocardial Infarction (REPAIR-AMI) trial (143) randomized 204 patients with acute MI to receive an intracoronary infusion of progenitor cells derived from bone marrow or placebo medium into the infarct artery 3–7 days after successful reperfusion therapy. The absolute increase in LVEF was significantly greater (2.5%) in the BMC group than in the placebo group at 4 months. However, other trials [ASTAMI-Autologous Stem Cell Transplantation in Acute Myocardial Infarction and STEMI (ST-elevation Acute Myocardial Infarction)] (73, 100) showed negative results with no improvement in ejection fraction with cell transplantation. Differences in cell preparation (148) and numbers have been proposed as possible causes for these conflicting results.

Bone marrow cells also were used in chronic heart failure setting. In the Transplantation of Progenitor Cells and Recovery of LV Function in Patients with Chronic Ischemic Heart Disease (TOPCARE-CHD) trial (9), 75 patients with stable ischemic heart disease who had had a myocardial infarction at least 3 months previously were assigned to receive either no cell infusion or infusion of circulating progenitor cells or BMCs into the patent coronary artery supplying the most dyskinetic left ventricular area. The transplantation of BMCs was associated with a moderate (2.9 percentage points) but significant improvement in left ventricular function 3 months after transplantation.

Mesenchymal stem cells

MSCs have features that make them attractive candidates for cell transplantation. MSCs can be isolated from the bone marrow by a simple process involving Ficoll centrifugation and adhering-cell culture in defined serum-containing medium. MSCs can be expanded for four to 20 population doublings only (133), with preservation of the karyotype, telomerase activity and telomere length (128, 131). MSCs could potentially become an "off-the-shelf" allogeneic product that would be more cost effective, easier to administer, allow a greater number of cells to be transplanted, and, possibly of importance, permit transplantation at the time of urgent interventions to relieve ischemia and injury, such as percutaneous or surgical revascularization procedures. Importantly, these cells appear to avoid rejection by being hypoimmunogenic (14, 89, 166). These cells lack MHC-II and B-7 costimulatory molecule expression and thus limit T-cell responses (141, 184). They can also directly inhibit inflammatory responses *via* paracrine mechanisms, including production of transforming growth factor β1 and hepatocyte growth factor (39, 88).

MSC transplantation has led to functional improvement in large-animal models of myocardial infarction. Allogeneic intramyocardial transplantation of MSCs in a porcine model of myocardial infarction resulted in profound improvement in border-zone energetic, regional, and global contractile function (180). This was thought to be related to paracrine mechanisms, as evidenced by increased vascularity in the border zone and spared native cardiomyocytes in the infarct zone (180). Percutaneous delivery of allogenic MSCs 3 days after myocardial infarction in a porcine model resulted in long-term engraftment at 8 weeks, profound reduction in scar size, and near normalization of cardiac function (5). MSC transplantation has been used in patients with acute myocardial infarction 18 days after primary percutaneous intervention and resulted in significant improvement in LVEF up to 6 months (24, 25). This was associated with a significant reduction in the size of the perfusion defect measured with positron emission tomography at 3 months (24, 25). More recently, it was demonstrated in a randomized double-blind placebo-controlled trial that intravenous delivery of allogenic human MSCs leads to improved ventricular function after myocardial infarction (178). The degree of response to IV therapy occurs early after MI and compared favorably with previous studies using intracoronary infusions of bone marrow (178).

Endothelial progenitor cells

Endothelial progenitor cells (EPCs) have been used in different animal models of cardiovascular disease. Intramyocardial implantation of $CD34^+$ selected human peripheral blood mononuclear cells into nude rats after myocardial infarction resulted in neovascularization and improved left ventricular function (77). The clinical application of EPCs is limited by the fact that it is difficult to expand them into sufficient numbers without inducing a change in phenotype or the development of cell senescence. Erb *et al*. (44) randomized patients with chronically occluded coronary arteries to receive intracoronary progenitor cells or placebo. They mobilized bone marrow cells by using GCSF, harvested them from peripheral blood, and expanded them *ex vivo*. The intracoronary delivery of these cells led to improvement in coronary flow reserve and cardiac function at 3 months after transplant. Currently, clinical trials using $CD34^+$ cells from bone marrow that are enriched in EPC content are under way.

Umbilical cord blood stem cells

Human umbilical cord blood (UCB) is rich in stem and progenitor cells, which have high proliferative capacity (92, 105, 115). Human UCB contains fibroblast-like cells termed unrestricted somatic stem cells, which adhere to culture dishes, are negative for c-kit, CD34, and CD45, and differentiate *in vitro* and *in vivo* into variety of tissue types, including cardiomyocytes (83). Direct intramyocardial injection of these human unrestricted somatic cells into the infarcted hearts of immunosupressed pigs resulted in improved perfusion and wall motion, reduced infarct size, and enhanced cardiac function (81). Further, intravenous injection of human mononuclear UCB cells, a small fraction of which were $CD34^+$, into NOD/scid mice led to enhanced neovascularization with capillary endothelial cells of both human and mouse origin and reduced infarct size (101). However, no myocytes of human origin were found, arguing against cardiomyogenic differentiation and regeneration of cardiomyocytes from donor cells. Additionally, direct intramyocardial

injection of UCB $CD34^{+}$ cells into the periinfarct rim in a rat model resulted in improved cardiac function (65). No clinical studies of UCB transplantation have been reported.

Cardiac stem cells

The ability of the cardiomyocytes to replicate has been a highly controversial topic. It is known that increases in cardiac mass in mammals during fetal life occur mainly because of cardiomyocyte proliferation. However, during the perinatal period, mammalian cardiomyocytes are known to withdraw from the cell cycle, thus limiting their ability to divide and increase in number (103, 140, 154). Thus, normal postnatal growth and adaptive increases in cardiac mass in adults as a result of the hemodynamic burden are achieved mainly through an increase in cell size known as hypertrophy (103, 140, 154). This belief was supported by the inability to identify mitotic figures in myocytes, as well as the observation that regions of transmural infarction evolved into essentially avascular, thin, collagenous scar. This paradigm has been dominant over the past 50-year period and views the heart as a postmitotic organ consisting of a predetermined number of parenchymal cells that is defined at birth and preserved throughout life until the death of the organ and organism. However, recent studies over the past few years challenged the concept of the heart being an organ incapable of regeneration. It has been shown that the human heart contains cycling myocytes undergoing mitosis and cytokinesis under

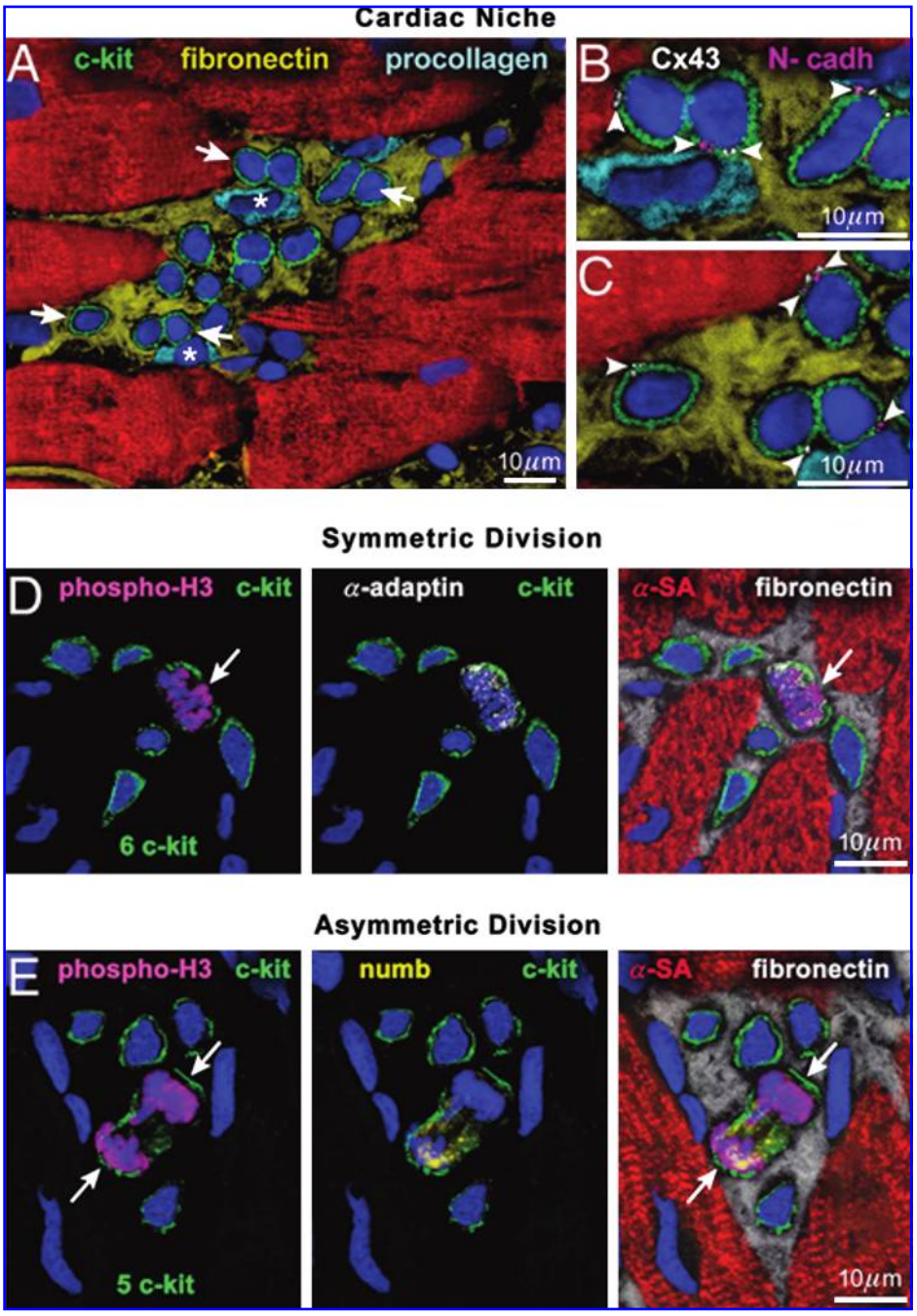

FIG. 2. Cardiac niches and human cardiac stem cell division. Sections of normal human myocardium. (**A–C**) Cluster of c-*kit*^{POS} cells (green). Arrows in **A** define the areas in **B** and **C**. Gap (connexin 43: Cx43, white; arrowheads) and adherens (*N*-cadherin: N-cadh, magenta; arrowheads) junctions are shown at higher magnification. Cx43 and N-cadh are present between c-*kit*^{POS} cells and myocytes (α-SA, red) and fibroblasts (procollagen, light blue); fibronectin, yellow. (**D** and **E**) Mitosis (phospho-H3, magenta; arrows) in c-*kit*^{POS} cells; α-adaptin (**D**, white) and Numb (**E**, yellow) show a uniform (**D**) and nonuniform (**E**) localization in the mitotic c-*kit*^{POS} cells. (Courtesy of P. Anversa and PNAS) (15). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article at www.liebertonline.com/ars).

normal and pathologic conditions (Fig. 2) (6, 17, 118, 134). This consequently means that cardiac progenitor cells in the heart can give rise to new myocytes. This raises the question that if the heart possesses progenitor cells capable of regenerating myocardium and forming new vessels, then why is the ischemic myocardium not spontaneously repaired and heart failure cured? This is probably related to the inability of the endogenous progenitor cells to home effectively to the infarct area. Moreover, the progenitor cells in the infarcted region do not survive the ischemic event and die by apoptosis or necrosis, as do other cardiomyocytes and vessels (168). These primitive cells cannot escape replicative senescence with severe telomeric shortening and activation of the death program in end-stage failure (169). Thus, by and large, tissue growth does not invade and replace the infarcted myocardium but is restricted to the noninfarcted portion of the ventricular wall (17, 167, 169). Cardiac progenitor cells (CPCs) can be isolated based on cell-surface stem cell markers c-kit or Sca-1, expression of islet-1 genes, ability to efflux Hoechst dye (SP, side population), and tissue culture of cardiac explants with spontaneous shedding of CPCs *in vitro*. Direct intramyocardial injection of c-kit$^+$ cells into an ischemic rat heart reconstituted well-differentiated myocardium, comprising blood-carrying new vessels and cardiomyocytes with the characteristics of young cells; these cells were present in approximately 70% of the ventricle (16). Later, it was also shown that intracoronary delivery of these cardiac stem cells in an ischemia/reperfusion rat model resulted in myocardial regeneration, infarct-size reduction of 29%, and improvement of left ventricular function (35). Given intravenously after ischemia/reperfusion, Sca-1 cells also homed to injured myocardium and differentiated into cardiomyocytes (120). The relative contributions of regenerated cardiomyocytes and preservation of injured native cardiomyocytes in these studies require clarification. CPCs and early committed cells (ECCs) express c-Met and insulin-like growth factor I receptors (IGF-IR) and synthesize and secrete the corresponding ligands, hepatocyte growth factor (HGF) and insulin-like growth factor-1 (IGF-1) (168). HGF mobilizes CSCs-ECCs, and IGF-1 promotes their survival and proliferation (168). Therefore, in another study, HGF and IGF-1 were injected in mice with myocardial infarction, and a growth factor gradient was introduced between the site of storage of primitive cells in the atria and the region bordering the infarct, to facilitate homing. The newly formed myocardium contained arterioles, capillaries, and functionally competent myocytes that increased in size over time. This regeneration was associated with improved ventricular performance and induced increased survival. Surprisingly, this intervention rescued animals with infarcts that composed 86% of the ventricular mass. These findings have been replicated in a dog model, in which HGF and IGF-1 were also used to stimulate resident cardiac stem cells after myocardial infarction; and growth factor therapy again resulted in improvement of myocardial function (97).

Before their therapeutic use, CPCs have to be isolated from fragments of myocardium and expanded *in vitro*. This was achieved in a pig model (15), in which c-kit$^+$ cells were isolated, and each cell was propagated to form approximately 400,000 cells. Another group performed autologous transplantation of CPCs in an ischemia/reperfusion swine model (74). Each pig had a biopsy from the right ventricular septum at the time of injury. The biopsies weighed 92 mg and yielded a mean cell counts of 14.2×10^6 cells after isolation and expansion (after 2.8 cell passages over a 23-day period). Intracoronary delivery was performed 4 weeks after injury. Engraftment occurred in the MI border zone, and islands of engrafted cells were present within the scar 8 weeks after coronary delivery (74).

Human cardiac progenitor cells also have been isolated from myocardium, expanded *in vitro* and then used for transplantation in animal models of ischemic myocardium. Hosoda *et al.* (68) isolated human cardiac progenitor cells (hCPCs) from surgical samples. These c-kit+ hCPCs were injected into the hearts of immunodeficient mice and rats. Foci of myocardial regeneration were identified at 2–3 weeks and consisted of myocytes, resistance arterioles, and capillaries (68). The presence of connexin 43 and *N*-cadherin in the developing human myocytes strongly suggested that the engrafted human cells were becoming functionally competent.

Two-photon microscopy was used to demonstrate further the functional integration of enhanced green fluorescent protein (EGFP)-positive human myocytes with the surrounding myocardium (68). Torella *et al.* (164) isolated hCPCs from myocardial samples from all four chambers of the human heart. These were c-kit$^+$, MDR-1$^+$, and CD133$^+$. One clone could generate more than 5×10^9 cells and form functional myocardium after injection into infarcted rat hearts (164). These studies provide a rationale for the use of human cardiac progenitor cells in patients with ischemic heart disease. These cells seem to be excellent candidates for exogenous stem cell therapy, but they have to be harvested from patients and expanded *ex vivo* to generate numbers sufficient for transplantation. No reported clinical trials of human cardiac progenitor cells have been undertaken.

Embryonic stem cells

Embryonic stem cells have the capacity to divide in an undifferentiated state while maintaining their ability to differentiate into cells belonging to all three embryonic germ layers. However, transplantation of these cells has certain limitations; they can form teratomas (163), are immunogenic (119), and ethical concerns regard their use.

Human ESCs were first isolated from the human blastocyst in 1998 (163), and later it was shown that they could differentiate into cardiomyocytes (80). The transplantation of these cells in the setting of myocardial infarction was limited by the fact that only 18% form myocardial grafts, and these grafts also contain substantial noncardiac elements (85). However, the differentiation of hESCs into cardiomyocytes can be enhanced by sequential treatment of high-density undifferentiated monolayer cultures with activin A and bone marrow morphogenic protein 4 (BMP4) (85). This protocol yielded $>30\%$ cardiomyocytes as opposed to $<1\%$ with the embryoid-body–based system, which used serum to induce differentiation (85). Furthermore, Percoll gradient centrifugation, which allows specific enrichment of hESC-derived cardiomyocytes, led to cultures of $82.6\pm6.6\%$ cardiomyocytes (85). Moreover, the graft survival in infarcted hearts can be improved by a prosurvival cocktail that includes Matrigel to prevent anikis, a cell-permeant peptide from Bcl-XL to block mitochondrial death pathways, cyclosporin A to attenuate cyclophilin D–dependent mitochondrial pathways, a compound that opens ATP-dependent K^+ channels (pinacidil) to

mimic ischemic preconditioning, insulin-like growth factor (IGF-1) to activate Akt pathways, and a caspase inhibitor, ZVAD-fmk (85). Transplantation of hESC-derived cardiomyocytes with this prosurvival cocktail in infarcted hearts caused consistent formation of myocardial grafts with improved ventricular function (85).

Enhancement of stem cell therapy in heart failure

Although stem cell transplantation improves left ventricular function after myocardial infarction, the observed stem cell engraftment is usually minimal. Furthermore, the majority of transplanted cells that do engraft remain as spindle-shaped stem cells and do not fully differentiate into host cardiac cell phenotypes. Therefore, other techniques are used to enhance the efficacy of stem cell transplantation (Fig. 3).

Stem Cell Mobilization

Granulocyte colony-stimulating factor (G-CSF), vascular endothelial growth factor (VEGF), stromal cell–derived factor-1 (SDF-1), angiopoeitin-1, placental growth factor, and erythropoietin are a few of the therapies used to mobilize stem cells from bone marrow to the systemic circulation to enhance endogenous repair and to facilitate collection of these cells for *ex vivo* expansion and use in cell therapy. As an example, intracoronary infusion of peripheral blood stem cells mobilized by G-CSF resulted in improvement of left ventricular function in patients with myocardial infarction (76).

Stem cell homing

It is extremely important to enhance the homing of stem cells to the injured region of the heart. Factors that contribute to homing of stem cells include stromal-derived growth factor (SDF-1) (8, 21), high-mobility group box protein 1 (HMGB1) (96), monocyte chemotractant protein 3 (MCP-3) (144), growth-related oncogene 1 (GRO-1) (82), hepatocyte growth factor (HGF), fibroblast growth factor 2 (FGF-2), and insulin growth factor (IGF) (168). Many of these factors are expressed in the setting of myocardial infarction to enhance endogenous stem cell homing; however, the duration of expression is probably limited. Engineering of MSCs or HSCs to overexpress SDF-1 receptor CXCR4 resulted in greater homing of engineered MSCs and improvement of left ventricular function compared with that of control MSCs or HSCs when the cells were delivered within 24 h of myocardial infarction (27, 75, 125).

Stem cell function and survival

Assuming that the number of transplanted cells that survive is critical to therapeutic benefit, multiple groups are working on methods to increase the survival of transplanted cells; a great deal of experimental evidence suggests that this strategy may be effective. Apoptosis can be decreased by constitutive expression of Akt (a serine threonine kinase with potent prosurvival activity) or by heat shock before transplantation (183). Rat MSCs transduced to overexpress Akt1 (encoding the Akt protein) transplanted into ischemic myocardium were found to inhibit cardiac remodeling by reducing inflammation, collagen deposition, and myocyte hypertrophy in a dose-dependent fashion (104). MSCs transduced to express Akt were also studied in an ischemic porcine model, which showed an improvement in EF as compared with that of nontransduced MSCs. To determine the mechanism of the beneficial effect, the effects of the apoptotic stimulus, H_2O_2, on MSCs transduced with Akt was studied *in vitro*. Akt-MSCs were found to be more resistant to apoptosis, and this was related to higher levels of extracellular signal-regulated protein kinase (ERK) activation and VEGF (95). Another strategy that has been widely tested involves attempts to increase vasculogenesis with VEGF. Transfection with VEGF and IGF-1 improved survival of transplanted bone marrow cells in a rat model of myocardial infarction (176). Delivery of cells that had undergone adenoviral transduction

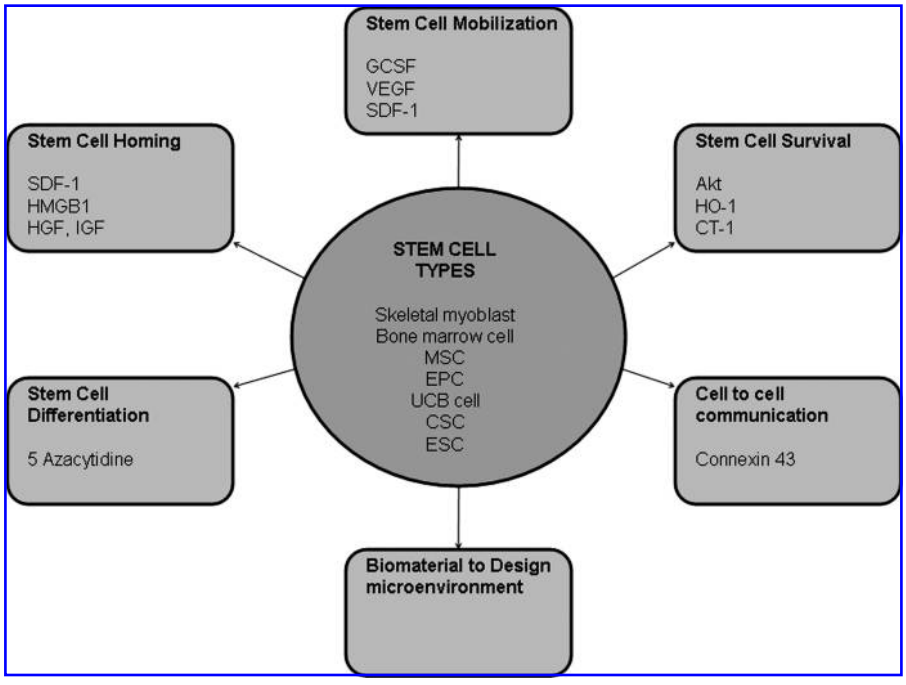

FIG. 3. Stem cell therapy for heart failure.

and overexpressed VEGF also resulted in improved LV function and neovascularization (7), but the addition of VEGF protein alone to cells did not show any benefit in a rat model of fetal cardiomyocyte transplantation (146). Transplantation of MSCs overexpressing SDF-1 also resulted in 60% decrease in cardiomyocyte apoptosis 4 days after MI, 150% increase in the number of surviving cardiomyocytes, and >200% increase in EF at 5 weeks (182).

Augmentation of the expressions of other gene products also has been tested and found effective, including cardiotrophin-1 (CT-1), heme oxygenase-1 (HO-1), an IL-1 inhibitor, and CuZn-superoxide dismutase. MSCs transfected with a hypoxia-regulated HO-1 vector are more tolerant to hypoxia-reoxygenation injury *in vitro* and result in improved viability in ischemic hearts (160). Treatment with CuZn-superoxide dismutase has been shown to attenuate the initial rapid cell death after transplantation, leaving a twofold increase in the total number of engrafted cells at 72 h compared with controls (157).

Cell-to-cell communication

Cardiac electromechanical coupling is the process that enables electrical excitation of cardiac cells and tissue to result in mechanical contraction of the cell and, hence, the tissues. Heart muscle cells are electromechanically coupled by specialized cell–cell junctions, the intercalated disks, which contain adherens and gap junctions for mechanical and electrical coupling, respectively. The major adherens junction protein in the mature mammalian heart is *N*-cadherin, whereas connexin-43 is the major gap-junction protein. Electromechanical coupling between cardiomyocytes is a basic requirement for coordinated mechanical activity in myocardium. Lack of communication between transplanted cells and the host myocardium predisposes to arrhythmias. The degree of arrhythmogenic potential after cell transplantation seems to correlate with the degree of connexin protein expression (112). Transplantation of skeletal myoblasts expressing connexin 43 resulted in decreased post-infarction ventricular tachycardia, as compared to control skeletal myoblasts (137). Despite major advances in the field, no evidence of complete electromechanical coupling has been noted after cell transplantation. No evidence exists that depolarization and repolarization together with contraction and relaxation of transplanted cells occurs in synchrony with native cardiac myocytes, despite evidence of connexin-43^{+} intercellular junctions and Ca^{2+} fluxes between cells.

Stem cell differentiation to cardiomyocytes

Potential strategies to enhance differentiation of stem cells into cardiomyocytes would help boost myocardial regeneration after transplantation. 5-Azacytidine treatment of MSCs enhances myogenic differentiation and leads to greater improvement in ventricular function, as compared with MSCs alone (20, 177).

Use of biomaterials to design microenvironment

The microenvironment in which the cells are injected is of extreme importance to their survival and subsequent beneficial effects. Biomaterials can be designed to regulate quantitative timed release of factors that direct cellular-differentiation pathways, such as angiogenesis and vascular maturation. Moreover, smart biomaterials even respond to the local environment, such as protease activity or mechanical forces, with controlled release or activation (33). Cardiac patches are three-dimensional matrices composed of natural or synthetic scaffold materials that can host the cells to allow longer cell viability and to enhance differentiation and integration. These patches may also contain factors that enhance neovascularization to allow the patch to survive in ischemic tissue. Zhang *et al.* (181) showed that controlled release of SDF-1 *in situ* increases stem cell homing to the infarcted heart. Lee *et al.* (34) designed self-assembling peptide nanofibers for prolonged delivery of IGF-1 to the myocardium, by using a "biotin sandwich" approach. Biotinylated IGF-1 was complexed with tetravalent streptavidin and then bound to biotinylated self-assembling peptides. After injection into rat myocardium, biotinylated nanofibers provided sustained IGF-1 delivery for 28 days, and targeted delivery of IGF-1 *in vivo* increased activation of Akt in the myocardium. Cell therapy with IGF-1 delivery by biotinylated nanofibers improved systolic function after experimental myocardial infarction, demonstrating how engineering the local cellular microenvironment can improve cell therapy.

Most of these new biomaterials provide much greater flexibility for regenerating tissues *ex vivo*, but emerging technologies, like self-assembling nanofibers, can now establish the intramyocardial cellular microenvironments by injection. This may allow percutaneous cardiac regeneration and repair approaches (*i.e.*, injectable-tissue engineering). Finally, materials can be made to multifunction by providing sequential signals with custom design of differential-release kinetics for individual factors. Thus, new rationally designed biomaterials no longer simply coexist with tissues, but can provide precision bioactive control of the microenvironment that may be required for cardiac regeneration and repair. One of the biggest challenges with using biomaterials is controlling the tissue reaction after implantation or injection. This response consists of an initial local injury, which can then initiate an inflammatory response and foreign-body reaction. Acute inflammation, which can last from minutes to days, involves migration of leukocytes into the tissue and results in edema formation. This can lead to chronic inflammation if persistent exposure to an inflammatory material occurs, characterized by presence of macrophages, monocytes, and lymphocytes. Blood vessels and connective tissue also begin to proliferate at this stage, with consequent development of granulation tissue within 3 to 5 days of implantation. Implantation of a biomaterial can also result in a foreign-body reaction characterized by foreign-body giant cell invasion. This leads to fibrous tissue formation and encapsulation. When biomaterials containing components of other species are used, more-specific antigen-mediated responses can also occur. An excessive immune response can even lead to hypersensitivity, which can result in tissue damage due to the release of intracellular chemicals, or excessive thrombus formation. Biomaterials are also prone to bacterial contamination and subsequent infection at the implant site, and thus the ability to sterilize a biomaterial properly also is critical.

Conclusions

Research into the molecular and pathophysiological mechanisms underlying heart failure has led to development

of new drugs and therapies for heart failure. These include gene and stem cell therapy, which have shown promising results. However, these therapeutic strategies require further preclinical research before successful clinical application.

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Abbreviations

AAV, adenoassociated virus; AC, adenylate cyclase; ACEI, angiotensin-converting enzyme inhibitor; AdV, adenovirus; Akt, a serine threonine kinase; ANP,atrial natriuretic peptide; AR, adrenergic receptor; ARB, angiotensin-receptor blocker; ASTAMI, Autologous Stem Cell Transplantation in Acute Myocardial Infarction; ATP, adenosine triphosphate; Bcl-2, B-cell lymphoma 2 (antiapoptotic gene); Bcl-XL, B-cell lymphoma XL (antiapoptotic gene); BM, bone marrow; BMC, bone marrow cell; BMMNC, bone marrow mononuclear cell; BMP, bone morphogenic protein; BNP, brain natriuretic peptide; BOOST, bone marrow transfer to enhance ST-elevation infarct regeneration; CAD, coronary artery disease; cAMP, cyclic adenosine monophosphate; CCS, Canadian Cardiovascular Society; c-Met, mesenchymal epithelial transition factor; CRT, cardiac resynchronization therapy; CUPID, Calcium-Upregulation by Percutaneous Administration of Gene Therapy in Cardiac Disease; CPC, cardiac progenitor cell; CT-1, cardiotrophin-1; CXCR4, chemokine receptor for stromal-derived factor-1; EC, excitation contraction; ECC, early committed cell; EcSOD, extracellular superoxide dismutase; EF, ejection fraction; EF-hand, helix–loop–helix structural domain in calcium-binding proteins; EGFP, enhanced green fluorescent protein; EndMT, endothelial–mesenchymal transition; EPC, endothelial progenitor cell; ERK, extracellular signal-regulated protein kinase; ESC, embryonic stem cell; FGF, fibroblast growth factor; GCSF, granulocyte colony-stimulating factor; GFP, green fluorescent protein; GRK, G protein–coupled receptor kinase; GRO-1, growth-related oncogene 1; HGF, hepatocyte growth factor; HIF, hypoxia-inducible factor; HMGB-1, high-mobility group box protein 1; HO-1, heme-oxygenase 1; H_2S, hydrogen sulfide; HSC, hematopoietic stem cell; ICD, intracardiac defibrillator; IGF, insulin-like growth factor; IGF-1R, insulin-like growth factor 1 receptor; IL-1, interleukin 1; IP, inhibitor protein; KAT, Kupio Angiogenesis Trial; KO, knockout; LV, left ventricle; LVAD, left ventricular assist device; MCP, monocyte chemoattractant protein; MI, myocardial infarction; MPT, mitochondrial permeability transition; MSC, mesenchymal stem cell; NO, nitrous oxide; NOD/scid, nonobese diabetic/severe combined immunodeficiency; NOS, nitrous oxide synthase; NO_2^-, nitrite; PKA, protein kinase 1; PLN, phospholamban; PP1, protein phosphatase 1; PTIO, 2-(4-carboxyphenyl)-4,4,5,5-tetramethylimidazole-1-oxyl 3-oxide; REPAIR-AMI, Reinfusion of Enriched Progenitor Cells and Infarct Remodeling in Acute Myocardial Infarction; ROS, reactive oxygen species; RyR, ryanodine receptor; SDF-1, stromal cell–derived factor 1; SERCA2a, SR Ca^{2+} ATPase in the myocytes; SP, side population; SR, sarcoplasmic reticulum; STEMI, ST-elevation acute myocardial infarction; TAC, transaortic constriction; TOPCARE-AMI, Transplantation of Progenitor Cells and Regeneration Enhancement in Acute Myocardial infarction; TOPCARE-CHD, Transplantation of Progenitor Cells and Recovery of LV Function in Patients with Chronic Ischemic Heart Disease; TGF, transforming growth factor; UCB, umbilical cord blood; VEGF, vascular endothelial factor.

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Address correspondence to:
Jianyi Zhang, M.D., Ph.D.
Department of Cardiology
University of Minnesota
420 Delaware Street SE
MMC 508
Minneapolis, MN 55455

E-mail: zhang047@umn.edu

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