Concise Review: Mending a Broken Heart: The Evolution of Biological Therapeutics

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ABSTRACT

Heart failure (HF), a common sequela of cardiovascular diseases, remains a staggering clinical problem, associated with high rates of morbidity and mortality worldwide. Advances in pharmacological, interventional, and operative management have improved patient care, but these interventions are insufficient to halt the progression of HF, particularly the end-stage irreversible loss of functional cardiomyocytes. Innovative therapies that could prevent HF progression and improve the function of the failing heart are urgently needed. Following successful preclinical studies, two main strategies have emerged as potential solutions: cardiac gene therapy and cardiac regeneration through stem and precursor cell transplantation. Many potential gene- and cell-based therapies have entered into clinical studies, intending to ameliorate cardiac dysfunction in patients with advanced HF. In this review, we focus on the recent advances in cell- and gene-based therapies in the context of cardiovascular disease, emphasizing the most advanced therapies. The principles and mechanisms of action of gene and cell therapies for HF are discussed along with the limitations of current approaches. Finally, we highlight the emerging technologies that hold promise to revolutionize the biological therapies for cardiovascular diseases. STEM CELLS 2017;35:1131–1140

SIGNIFICANCE STATEMENT

Innovative therapies that could treat heart failure and improve the function of failing hearts are urgently needed. Following successful preclinical studies, two main strategies have emerged as potential solutions: cardiac gene therapy and cardiac regeneration through stem and precursor cell transplantation. Many gene- and cell-based therapies have entered into clinical studies, intending to ameliorate cardiac dysfunction in patients with advanced HF. In this review, we focus on the recent advances in cell- and gene-based therapies in the context of cardiovascular disease, emphasizing the most advanced therapies that have entered the clinical arena.

INTRODUCTION

Cardiovascular diseases are debilitating and often deadly, responsible for more deaths worldwide than any other disease [1]. Specifically, heart failure (HF)—the common sequela of many cardiovascular diseases—affects over 38 million people, 50% of which are estimated to die within 5 years of diagnosis [2]. Given the limited intrinsic regenerative potential of the adult heart [3], the endogenous sources of regeneration and repair are insufficient to halt the progression of HF. In end-stage cases, the last available option for treatment is cardiac transplantation, which is limited by the shortage of organs available. Pharmacological and device-based treatments that are currently implemented into HF treatment guidelines have improved patient survival [4, 5]. Although these therapies are beneficial, they are limited in relieving symptoms and do not address the molecular mechanisms underlying the pathogenesis of HF and hence cannot reverse or slow the adverse remodeling of the heart. This limitation in available remedies has raised the need for therapies that could repair or even regenerate the injured myocardium. Biological therapies, such as gene- and cell-based approaches, have emerged as alternative therapies to treat both acute cardiac events such as myocardial infarction (MI) and chronic cardiovascular diseases, representing a new generation in biological therapeutics for HF.

In this review, we discuss the progress in the gene therapy field and the use of stem and precursor cells for stimulating endogenous regeneration and/or as a source for cardiomyocyte renewal, emphasizing the recent human clinical trials. In addition, we highlight

the biological processes that underpin the reported therapeutic benefits, and discuss the shortcomings, challenges, and future perspectives of gene- and cell-based therapies for HF. Finally, we examine the most exciting advances in the field, which hold promise as alternative approaches to cardiac regeneration and repair.

PROGRESS AND CHALLENGES IN STEM CELL CARDIAC REGENERATIVE THERAPY

Over the past two decades, substantial progress has been made in the field of stem cell therapy for cardiac repair. Despite the absence of an understanding of the mechanism through which donor cells improve cardiac function in patients, research has proceeded rapidly from preclinical models to clinical studies. To date, a plethora of relatively small clinical trials have tested the potential benefit of various cell types in patients with HF (Table 1).

BONE MARROW-DERIVED CELLS

Bone marrow consists of hematopoietic stem cells (HSCs) and nonhematopoietic multipotent cells, such as mesenchymal stem cells (MSCs), which can be induced to differentiate into the adipocytic, chondrocytic, or osteocytic lineages [28]. Bone marrow mononuclear cells (BMMNCs) can be readily obtained from patients by bone marrow aspiration and density gradient centrifugation without the need for culture in vitro before administration.

Since its inception over 15 years ago [29], the use of autologous BMMNCs as cell therapy for HF has been investigated in multiple randomized and nonrandomized trials, yielding conflicting and controversial clinical outcomes. Early clinical trials such as TOPCARE-CHD [15], REPAIR-AMI [9], and FINCELL [10] reported improved systolic function in treated acute MI (AMI) patients, while others reported either no significant improvements (ASTAMI, Leuven-AMI) [7, 8] or absence of any long-term benefits (BOOST) [6]. More recent trials with larger cohorts that were adequately controlled (FOCUS-CCTRN, TIME, Late TIME, REGENERATE-AMI) [11–14] found modest or no effect of BMC therapy on ventricular function and prespecified endpoints. Overall, all trials failed to show any improvements in clinical outcomes in the treated patients.

In an attempt to improve the therapeutic potential of autologous bone marrow cells, multiple trials have assessed the safety and efficacy of selected and ex vivo expanded subpopulations, such as MSCs. The C-CURE [18] trial was one of the first studies that implemented the concept of delivering cardiac lineage primed bone marrow-derived MSCs (termed "cardiopoietic stem cells") before myocardial implantation in patients with ischemic cardiomyopathy. This cardiopoietic cell population was derived by exposure of MSC (CD105, CD166, CD29, and CD44 and negative for CD14, CD34, and CD45) to a growth factor cocktail, including transforming growth factor-β, bone morphogenetic protein, activin A, fibroblast growth factor 2, cardiotrophin, and α-thrombin, which triggers hallmark traits of cardiac development [30]. Despite inconsistencies in the reported data [31], the study suggested that patients who received cells showed evidence of improved function versus

the control arm 6 months after treatment, suggesting that treatment with cytokine-primed MSCs is safe and feasible with signs of benefit in chronic ischemic HF. Another autologous bone marrow-derived subpopulation, termed ixmyelocel-T, has been tested in clinical trials in HF patients. Ixmyelocel-T is an expanded population of mesenchymal stromal cells and M2-like macrophages, as well as many of the $CD45^+$ cells found in the bone marrow. Although the precise mechanism of action is unknown, it is hypothesized that this expanded multicellular product induces tissue remodeling, immunomodulation, angiogenesis, and endothelial protection [32, 33]. The early phase, open label clinical trials (IMPACT-DCM and CATHETER-DCM) suggested that intramyocardial delivery of ixmyelocel-T might improve clinical, functional, symptomatic, and quality-of-life outcomes in patients with HF due to ischemic dilated cardiomyopathy (DCM) [16]. More recently, the phase IIB randomized, double-blind ixCELL-DCM [17] study showed that this multicellular therapy resulted in a significant reduction in adjudicated clinical cardiac events compared with placebo leading to improved patient outcomes, corroborating the findings of early trials.

The widespread use of BMMNCs can be attributed to immediate availability from the patient. Nonetheless, allogeneic cells could provide an even more readily available "off-the-shelf" therapeutic agent, avoiding the need for bone marrow aspiration and tissue culture delays before treatment. As such, allogeneic BM-derived MSCs have recently emerged as the leading candidate for an "off-the-shelf" product for HF cell-based therapy [34]. MSCs are considered immune-privileged and can be expanded in quantities unattainable from an autologous source, undergo cryopreservation, and be available for delivery. The early-stage study (POSEIDON) [21] was the first to demonstrate that alloimmune reactions in patients receiving allogeneic MSCs for ischemic left ventricle/ventricular (LV) dysfunction were low, suggesting that allogeneic MSC transplantation might be accomplished without the need for significant host immunosuppression. The trial reported similar safety profiles between the autologous and allogeneic MSCs. Although it was not powered to show efficacy, the MSC treatment favorably affected ventricular remodeling of patients with ischemic cardiomyopathy. Similarly, immunoselected bone marrow-derived mesenchymal precursor cells (MPCs), an enriched $Stro-1/Stro-3^+$ population, were evaluated in a phase II, multicenter, dose escalation study to determine feasibility and safety of three doses in patients with chronic HF. This study concluded that the high-dose allogeneic MPC treatment may reduce adverse cardiovascular events and provide beneficial effects on adverse left ventricular remodeling [20]. Taken together these studies suggest that allogeneic immune-selected MSCs are safe and potentially beneficial in treating patients with ischemic cardiomyopathy, offering an off-the-shelf readily available cell product. This beneficial effects attributed to multiple mechanisms have been proposed, including transdifferentiation, paracrine factor secretion with antiapoptotic, proangiogenic, and possibly immunomodulatory effects. However, to date, the precise mechanisms involved in the positive impact of MSCs remain to be identified.

Despite rapid clinical translation and widespread enthusiasm, the therapeutic benefits of bone marrow-derived cell (BMC) therapy in patients with heart disease remains

Table 1. Cell therapy clinical trials

Cell type	Phase	Name	Patient number	Condition	Treatment outcomes	Clinical trial identifier	References
BMMNC	I	BOOST	60	5–7 days post-MI	Improvement of LV systolic function after 6 months; no significant benefit after 18 months	NCT00224536	[6]
	II	Leuven-AMI	67	1 day post-MI	No significant effect on recovery of global LV function	NCT00264316	[7]
	II	ASTAMI	50	5–7 days post-MI	No improvement in global LVEF after 6 months	NCT00199823	[8]
	III	REPAIR-AMI	204	3–6 days post-MI	Significant increase in LVEF, reduced adverse events after 1 year	NCT00279175	[9]
	II/III	FINCELL	80	2–6 days post-MI	Improvement of global LVEF after 6 months	NCT00363324	[10]
	II	REGENERATE-AMI	100	1 day post-MI	Not significant improvement in LVEF after 1 year	NCT00765453	[11]
	II	TIME	120	3 or 7 days post-MI	No significant effect on recovery of LV function	NCT00684021	[12]
	II	Late-TIME	87	14–21 days post-MI	No significant improvement in LV function after 6 months	NCT00684060	[13]
	II	FOCUS-CCTRN	153	CAD	No significant improvement in LV volume, oxygen consumption or defect	NCT00824005	[14]
	III	BAMI[a]	3,000	AMI		NCT01569178	
	II	TOPCARE-CHD	75	MI	Significant increase in LVEF	NCT00289822	[15]
CD90+ MSC and CD45+ CD14+ Macrophages (Ixmyelocel-T)	IIa	IMPACT-DCM	39	DCM	Improves symptoms in patients with ischemic DCM	NCT00765518	[16]
	IIa	CATHETER-DCM	22	DCM	Improved symptoms in patients with ischemic DCM	NCT01020968	[16]
	II	IxCELL-DCM[a]	109	DCM	Reduced cardiac events with treatment	NCT01670981	[17]
Bone marrow-derived mesenchymal cardiopoietic cells	II/III	C-CURE	47	HF	Improved LVEF and quality of life	NCT00810238	[18]
	III	CHART-1[a]	240	HF		NCT01768702	[19]
	III	CHART-2[a]	240	HF		NCT02317458	
Allogeneic MPC Stro-1/Stro-3+	II		60	HF	High-dose significantly reduced adverse cardiac events	NCT00721045	[20]
	III	DREAM-HF[a]	600	HF		NCT02032004	
Autologous and allogeneic MSC	I/II	POSEIDON	30	HF	Improved functional capacity, quality of life, ventricular remodeling	NCT01087996	[21]
CSC	I	SCIPIO	33	HF	Increased LVEF and decreased infarct size	NCT00474461	[22, 23]
CDC	I	CADUCEUS	31	HF	No significant improvement on LVEF or scar reduction	NCT00893360	[24, 25]
	I/II	ALLSTAR[a]	134	MI		NCT01458405	
	I	DYNAMIC[a]	42	DCM		NCT02293603	
CDCs with bFGF	I	ALCADIA	7	HF	Safe and effective in patients with ischemic cardiomyopathy	NCT00981006	[26]
CSC + MSC	II	CONCERT-HF[a]	144	HF		NCT02501811	
ESCs CD15+ Isl-1+	I	ESCORT[a]	6	HF	No major complications after 3 months	NCT02057900	[27]

Abbreviations: AMI, acute myocardial infarction; bFGF, basic fibroblast growth factor; BMMNC, bone marrow mononuclear cell; CAD, coronary artery disease; CDC, cardiosphere-derived cell; CHD, coronary heart disease; CSC, cardiac stem cell; DCM, dilated cardiomyopathy; EF, ejection fraction; ESC, embryonic stem cell; HF, heart failure; LV, left ventricle/ventricular; MI, myocardial infarction; MPC, mesenchymal precursor cell; MSC, mesenchymal stem cell.
[a]Ongoing.

controversial. Differences in cell types, cell preparation standards, delivery techniques, imaging methods, and patient profiles can lead to incorrect inferences, and the effects of the therapies are difficult to interpret. Systematic review and meta-analysis of data from eligible randomized controlled trials could be informative, but have also yielded conflicting results, highlighting the lack of consistent efficacy in cell-based cardiac regeneration therapies [35–37]. Unexplained

discrepancies in design, methods, or results in many of the early phase clinical trials have also raised concerns over the validity of the reported benefits of bone marrow stem cell therapy [38]. It is apparent that only well-designed and adequately powered trials will establish whether BMC therapy offers a new hope to patients with HF. A series of studies have been designed as phase III confirmative randomized controlled clinical trials, including the BAMI trial (NCT01569178; http://www.bami-fp7.eu) a mortality trial enrolling 3,000 patients post-AMI throughout the European Union; the CHART-1 trial [19], which successfully enrolled 240 high-risk patients with advanced congestive HF; and the DREAM-HF study (NCT02032004), with a target enrollment of more than 600 high-risk patients with congestive HF have been designed as phase III confirmative trials. These studies are the most scientifically rigorous human experiments to date in the field of cardiac cell therapy. It is anticipated that the results of these clinical trials will be crucial in establishing whether BMC therapy represents an effective strategy for HF treatment.

ENDOGENOUS CARDIAC STEM CELLS

Recent findings have refuted the long-held belief that the adult mammalian heart is a terminally differentiated organ. There is, in fact, a constant cardiomyocyte turnover within human hearts throughout life, although at a very low rate [3]. Although the mechanisms of endogenous heart regeneration remain highly debatable, the discovery of putative resident cardiac stem cells (CSCs), such as c-kit$^+$ cells [39], provided the rationale that these cells could be isolated and harnessed to regenerate the failing heart [40]. Despite discrepant results, a plethora of preclinical studies demonstrate beneficial effects of c-kit$^+$ cell administration to ischemically damaged hearts despite the observed paucity of cardiomyogenic differentiation of these cells. The phenotype of postnatal c-kit$^+$ cardiac cells resembles traditional MSCs, suggesting their major mechanism of action involves paracrine actions [41].

The SCIPIO trial was the first human, randomized, open-label trial of autologous c-kit$^+$ CSCs in patients with ischemic HF undergoing coronary artery bypass grafting [22]. The initial results of the study showed a striking improvement of LV function and decreased infarct size at 4 months and 1 year after intracoronary infusion [22, 23]. However, concerns regarding the integrity of the published data have been raised, casting doubts over the validity of the study [42]. Another potential source for cardiac-derived stem cell therapy is cardiosphere derived cells (CDCs), a heterogeneous mixture of many different cell types derived by *ex vivo* culture of right ventricular endomyocardial biopsies [43]. The enhanced potency of cardiospheres for myocardial repair has been attributed to their growth properties that mimic stem cell niche properties with enhanced "stemness" and expression of ECM and adhesion molecules [44]. The CADUCEUS trial was a proof-of-concept study that evaluated the safety and efficacy of autologous CDCs in patients with a recent MI. The results showed no significant difference in heart function, end-systolic, or end-diastolic volumes with the treatment, but analysis of exploratory efficacy endpoints revealed an increase in viable myocardium after 6 and 12 months, suggestive of therapeutic cardiac regeneration [24, 25]. In addition, the

ALCADIA [26] study tested a novel approach of combining CDCs with a hydrogel-based delivery method of basic fibroblast growth factor (bFGF) [45] in patients with advanced HF. The interim results of the study demonstrated that the combination of CDCs and bFGF is safe, but given the small size of the study and the absence of a control group, no conclusion be drawn regarding the safety and the efficacy of this approach.

The aforementioned proof-of-concept studies have the potential to revolutionize the treatment of HF. However, the small number of enrolled patients, the short period of follow up, and the preliminary nature of the findings preclude any safe conclusions. In addition, although the c-kit$^+$ CSCs and CDCs are cardiac-derived cells that have been named "cardiac progenitor cells," there is no compelling evidence that they can differentiate into myocardial cells, and therefore the mechanisms involved in the beneficial actions observed remain unknown. Despite these uncertainties, prospective, randomized, placebo-controlled clinical trials are currently investigating the efficacy of cardiac-derived cell therapies in HF. For example, the DYNAMIC (NCT02293603) and ALLSTAR (NCT01458405) trial are currently evaluating the safety and efficacy of allogeneic CDCs in patients with DCM and MI, respectively. Similarly, a hybrid cell therapy composed of autologous c-kit$^+$ CSCs and bone marrow-derived MSCs is currently being tested in the CONCERT-HF, a phase II trial (NCT02501811) in patients with ischemic cardiomyopathy. The results of these promising studies are eagerly awaited.

EMBRYONIC STEM CELLS

Pluripotent stem cells (PSCs)—either embryonic stem cells (ESCs) or induced pluripotent stem cells (iPSCs)—have emerged as a renewable cell source for heart regenerative applications [46]. Human PSCs are attractive because they can be differentiated with great efficiency into cardiomyocytes (CMs) [47], providing an unlimited supply of cardiomyocyte-like cells in vitro, before transplantation. After a long period of preclinical and translational work, the first human trial, ESCORT [27], was initiated in 2013 with the first patient receiving purified ESC-derived cardiac progenitors (CD15$^+$ Isl-1$^+$) in an epicardial fibrin gel patch. Although the preliminary results suggested an improvement in the kinetics of the non-bypassed cell-patched area at 3 months and 6 months follow up, it would be meaningless to draw any conclusions regarding the efficacy of this treatment based on a single patient. This landmark study demonstrated the feasibility of producing clinical-grade ESC-derived cardiac progenitor cells and represents the first clinical application of this approach in the setting of HF, but additional studies are necessary to evaluate its safety and efficacy. Although the capacity of ESCs to differentiate toward the cardiac lineages is well established, numerous challenges remain for the clinical implementation of ESC-based therapies [48, 49]. For example, allogeneic ESCs face immunological challenges that might require life-long immunosuppression; theoretically autologous iPSC-derived cardiomyocytes circumvent this issue. PSC derivatives pose the inherent risk of forming teratomas. In addition, cell survival, retention, and engraftment are major obstacles. Even when cells successfully engraft and survive in the injured heart,

Table 2. Gene therapy clinical trials

Molecular target	Delivery mode	Phase	Name	Patient number	Treatment outcomes	Clinical trial identifier	References
VEGF	Adenovirus	II	KAT	103	Significant increase in myocardial		[52]
	Plasmid	I	VIVA	178	perfusion		[53]
	Adenovirus	I	KAT301	30	Enhanced myocardial perfusion	NCT01002430	[54]
	Plasmid	III	EUROINJECT-ONE	80	No difference in myocardial perfusion		[55]
	Plasmid	II/III	NORTHERN	93		NCT00143585	[56]
	Adenovirus	III	REVASC	17			[57]
FGF4	Adenovirus	II/III	AGENT-3	416	No beneficial effect	NCT00346437	[58]
		II/III	AGENT-4	116		NCT00185263	
		I	AGENT	79	Trend for improved myocardial		[59]
		I	AGENT-2	62	perfusion		[60]
AC6	Adenovirus	I/II	AC6 Gene Transfer	56	Dose-related improvement of cardiac function	NCT00787059	[61]
SERCA2a	AAV1	I/II	CUPID	51	Decreased HF symptoms remodeling	NCT00454818	[62–65]
		II/III	CUPID-2b	250	No improvement in the clinical course of HF	NCT01643330	[66]
		II	AGENT-HF[a]	10		NCT01966887	
		II	SERCA-LVAD[a]	5		NCT00534703	
SDF1	Plasmid	I	ACRX-100	17	Improvements 6-minute walk	NCT01082094	[67]
		II	STOP-HF	90	Improvements 6-minute walk	NCT01643590	[68]
		IIb	STOP-HF2[b]	180			

Abbreviations: AAV, adeno-associated virus; AC6, adenyl cyclase 6; FGF, fibroblast growth factor; HF, heart failure; LV, left ventricle/ventricular; SDF1, stromal-derived factor 1; SERCA2a, sarcoplasmic reticulum Ca^{2+} ATPase; VEGF, vascular endothelial growth factor.
[a]Terminated.
[b]Ongoing.

PSC-derived CMs could potentially trigger malignant arrhythmias, due to the presence of phenotypically immature cells with spontaneous beating activity [50]. Larger preclinical studies investigating cell dose, timing, and delivery modalities using consistent and efficient methods are necessary to address the aforementioned concerns and to conclusively demonstrate that the PSC-derived CMs and/or cardiomyocyte progenitor cells can improve cardiac function.

In summary, stem cell/progenitor cell therapies have been rapidly translated from bench to bedside, and numerous clinical trials have been spurred over the last 15 years. The initial enthusiasm generated by early-stage studies has now been met with skepticism, as the clinical outcomes of most BMC-based trials have yielded inconclusive results. Similarly, putative heart-derived stem cells, such as c-kit[+] and CDCs, have been proposed as attractive candidates for heart regeneration, but their therapeutic value remains questionable. Notably, it is not clear whether the modest beneficial effects are cell-type specific and the mechanisms of cardioprotection have not been completely unraveled yet. With the exception of PSC-derived CM progenitor cells, BMCs, MSCs, CDCs, and CSCs do not represent bona fide stem cell populations and are unlikely to regenerate the myocardium. Originally hypothesized to differentiate into new CMs, the aforementioned cell types are now known to engraft poorly, with the majority persisting less than a week after transplantation. Regardless of the cell source, the current consensus is that the transplanted cells do not generate new tissue, and it has been postulated that their beneficial effect is exerted via paracrine mechanisms that stimulate the endogenous repair pathways through the release of various factors. Nevertheless, these paracrine mechanisms have yet to be elucidated, and studies to determine exact mechanisms of action in the diseased human heart are needed to develop more targeted and robust cell therapies. Despite these mechanistic uncertainties, it is important to acknowledge that the safety and feasibility of BMCs, MSCs, CDCs, and CSCs has been consistently established. Recent advances with PSCs hold promise for successful clinical translation, but it will take time to develop effective and safe protocols for the use of PSCs in heart failure. The completion of the ongoing Phase II/III studies will certainly contribute knowledge and most likely provide valuable information to the cardiac regenerative medicine conundrum.

PROGRESS AND CHALLENGES IN GENE THERAPY FOR CARDIOVASCULAR DISEASE

Over the past decade, our understanding of the complex disease mechanisms underlying the pathogenesis of HF has significantly improved [51], and advances in molecular cardiology have identified key targets within the progression of HF. Gene therapy has emerged as a viable therapeutic strategy for specifically modulating underlying disease mechanisms, potentially replacing the symptomatic approach of existing treatments. Rectifying the disease at the gene level could mean a more permanent therapeutic benefit that could slow down or even reverse the detrimental course of HF. Extensive investigation into new treatment modalities has led to the development of gene-based therapeutic interventions, and in recent years there have been rapid advancements in gene therapy for HF (Table [2–68, 72, 75, 76]).

Gene therapy was proposed to be particularly valuable in the context of coronary artery disease (CAD), the most common type of cardiovascular disease. Preclinical studies have shown that a number of growth factors including vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), hepatocyte growth factor (HGF), platelet-derived growth factor (PDGF), and hypoxia-inducible factor (HIF) could

promote angiogenesis and induce vascular permeability and cytoprotective effects. Most gene therapy clinical trials for CAD were focused on the administration of genes encoding angiogenic growth factors, such as VEGF and FGF4, aiming to promote the development of collateral blood vessels in ischemia-related conditions. Early trials, such as VIVA [53] and KAT [52], suggested a functional improvement in myocardial perfusion and cardiac function in patients with CAD after the administration of either an expression plasmid or an adenoviral vector expressing VEGF-A$_{165}$, respectively. However, subsequent, double-blind, randomized, placebo-controlled studies (EUROINJECT-ONE, NORTHERN) [55, 56] failed to demonstrate any improvement in myocardial perfusion. Similarly, the phase II randomized, controlled REVASC [57] trial that evaluated the efficacy of an adenoviral vector-mediated VEGF delivery (Ad.VEGF-A$_{121}$) did not show any significant improvement in the primary endpoint of myocardial perfusion and alleviation of symptoms. Recently, another member of the VEGF family, VEGF-D, has also been clinically evaluated in patients with severe CAD. The preliminary data of the KAT301 trial [54], a randomized, placebo-controlled, single-blinded phase I/II study, suggest that adenoviral-mediated VEGF-D gene therapy is safe and could enhance myocardial perfusion. In an alternative approach, early phase I/II trials demonstrated that FGF4 treatment improved exercise capacity and reduced ischemic defect size in CAD patients (AGENT and AGENT-2) [59, 60]. However, the larger phase III trials (AGENT-3 and AGENT-4) [58] failed to corroborate these benefits. Consequently, both studies were terminated after an interim analysis of the AGENT-3 trial indicated that there were no significant differences regarding the primary endpoint in the between the treatment and placebo groups.

In advanced HF, cardiac calcium (Ca^{2+}) cycling—the release and reuptake of intracellular Ca^{2+} that drives muscle contraction and relaxation—is profoundly altered, resulting in impaired contractility and fatal cardiac arrhythmias [69]. Key components of the machinery that regulates Ca^{2+} cycling in the heart have emerged as prominent targets for human HF therapy [70, 71]. Notably, heart failure is associated with depressed sarcoplasmic reticulum (SR) calcium cycling, reflecting impaired SR Ca^{2+} transport and Ca^{2+} release, which has been attributed to reductions in gene and protein expression, or activity of the SR Ca^{2+} uptake pump (SERCA2a). SERCA2a plays a key role in transporting Ca^{2+} from the cytosol into the lumen of the SR following cardiac contraction, thus regulating cardiac contractility and relaxation. Targeting SERCA2a showed beneficial results in preclinical testing, leading to the first-in-human trial to enhance SR Ca^{2+} uptake in 2007 [62, 63]. In this phase I/II trial (CUPID), a small number of patients with advanced HF received an intracoronary administration of an adeno-associated viral (AAV1) vector expressing *SERCA2a* (AAV1.*SERCA2a*) and later showed improvements in key clinical outcomes [64]. Although individual patients did not show improvements across all parameters, improvements in prespecified primary endpoint criteria were observed in the highest dose cohort [65]. The promising outcome of the initial trial led to a larger Phase IIB, double-blind, placebo-controlled study, CUPID2, and two smaller auxiliary studies, AGENT-HF and SERCA-LVAD trials. The recently completed CUPID2 study did not meet its primary or secondary endpoints, and overall failed to demonstrate any improvement of clinical outcomes

in patients with advanced HF [66] Following the outcome of the CUPID2 trial, patient enrollment in both AGENT-HF and SERCA-LVAD studies was suspended. Importantly, no safety issues emerged—from the procedure of delivering the virus or long-term effects—in the participant patient population at the tested AAV1 therapeutic dose.

Adenylyl-cyclase type 6 (AC6) is an enzyme that serves as the effector molecule for β-adrenergic signaling, playing a key role in contractile responsiveness, cardiac relaxation, and LV diastolic function [72]. In preclinical studies, adenoviral-mediated delivery of an AC6 transgene improved LVEF and increased survival rates in animal models of cardiomyopathy in part due to increased SERCA2a activity and improved Ca^{2+} handling in CMs [73, 74]. A recent randomized, double-blinded, placebo-controlled clinical trial evaluated the safety, tolerability, and clinical effectiveness of ascending doses of adenovirus-5 encoding human AC6 (Ad5.hAC6) in patients with stable but severe HF [61]. Although the rates of serious adverse events were similar in both groups, the findings of this small clinical study suggest that intracoronary delivery of Ad5.hAC6 in patients with HF appears to be safe with a dose-related improvement in cardiac function at 4 and 12 weeks after randomization. The size of the study, however, was too small to draw any definitive conclusions regarding the efficacy and long-term benefit of this promising new gene therapy target in patients with advanced HF.

The stromal cell-derived factor-1 (SDF-1), and its receptor, chemokine receptor type 4 (CXCR4) has emerged as a key regulator in endogenous tissue repair. Preclinical studies indicate that a SDF-1 promotes tissue repair through the SDF-1:CXCR4 axis by promoting cell survival, endogenous stem cell recruitment, and vasculogenesis [75, 76]. The safety and potential efficacy of SDF-1 gene therapy was initially demonstrated in an open-label Phase I study in patients with ischemic cardiomyopathy [67]. According to the results of the more recent STOP-HF trial [68], the transient overexpression of SDF-1 has the potential to improve cardiac function in patients with ischemic cardiomyopathy. Although the trial failed to meet its primary efficacy endpoint, a prespecified subanalysis demonstrated that the potential benefits were more pronounced in patients with advanced cardiac dysfunction for at least 1 year post-treatment. These promising findings have led to the design of a larger, prospectively designed clinical study (STOP-HF2) that is expected to enroll up to 180 HF patients.

In short, despite extensive preclinical evaluation and encouraging results from early clinical studies, to date none of the gene therapy approaches have provided compelling evidence of a significant clinical benefit in HF patients. Early studies that focused on neovascularization have shown limited efficacy and consequently the angiogenic gene-therapy approaches with the goal to improve cardiac vascularization have largely been abandoned. More recently, the first human clinical trial of viral vector-based gene transfer for advanced HF was initiated after pilot clinical trials targeting the Ca^{2+} cycling pathway in HF patients showed favorable clinical outcomes without safety issues. However, the milestone CUPID2 trial failed to meet its prespecified endpoints, demonstrating that establishing clinical efficacy of novel therapeutic principles is a long and arduous path.
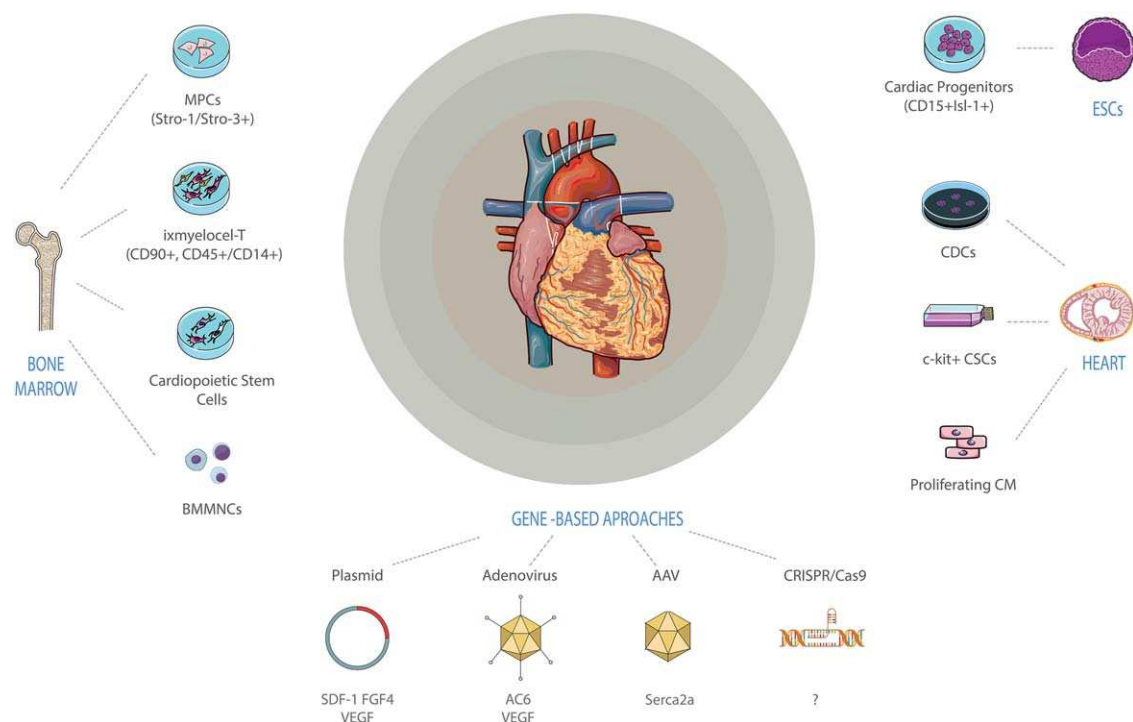
Figure 1. Biological therapeutics for the treatment of heart failure. Sources of stem cells used for cardiac repair include a broad range of bone marrow-derived stem cells, resident cardiac stem cells, and embryonic stem cell-derived progenitors. Current gene therapy approaches use DNA plasmids, adenoviruses, adenovirus-associated viruses, the application of new genome editing tools, such as the clustered regularly interspaced short palindromic repeats (CRISPR)-associated nuclease 9 (Cas9), holds great promise for gene therapy. Abbreviations: AC6, adenyl cyclase 6; BMMNC, bone marrow mononuclear cells; FGF, fibroblast growth factor; MPC, mesenchymal precursor cell; SDF-1, stromal-derived factor 1; VEGF, vascular endothelial growth factor. ESCs, embryonic stem cells; CSCs, cardiac stem cells; CDCs, cardiosphere derived cells; CM, cardiomyocyte.

CONCLUSION AND FUTURE DIRECTIONS

HF, a complex clinical syndrome, represents a major global health problem. Significant progress has been made over the past two decades in cell- and gene-based therapies for HF, promising the development of innovative therapeutic strategies for both treatment and prevention (Fig. 1). There are, of course, substantial gaps in knowledge that pose obstacles to the realization of the full potential of such novel biological therapies for clinical benefit. There is still a tremendous amount of work to be done, especially in addressing the need for deeper insights into the underlying disease mechanisms (i.e., which cell types, which genes, and at what levels, which pathways are relevant to any given pathogenic process, and which patients to treat).

Perhaps one of the most promising developments in the field of the regenerative cardiology is the emerging notion of using pre-existing cardiomyocytes as the source for cardiomyocyte replacement to maintain normal myocardial homeostasis as well as after myocardial injury [77–80]. The stimulation of proliferation of pre-existing cardiomyocytes could provide new avenues for future therapeutic strategies to regenerate the heart. However, further evidence and characterization for this putative pool of cycling cardiomyocytes as well as development of the means of therapeutic manipulation is a prerequisite to harness the endogenous regenerative properties of the adult heart.

Finally, genome-editing tools such as programmable engineered nucleases [81] are becoming more accessible [82] and are being used to increase our understanding of disease mechanisms as well as to develop novel therapeutic approaches. Gene correction by genome editing has shown great promise for clinical translation, as highlighted by recent studies for the treatment of Duchenne Muscular Dystrophy in vivo [83–85] and DCM in vitro [86]. Nevertheless, these novel approaches will likely have to address the problem of delivery that has been a key issue in gene therapeutic strategies targeting the heart. Although the genome editing field is in its infancy, these studies represent an important step towards the treatment of hereditary forms of cardiovascular diseases.

AUTHOR CONTRIBUTIONS

C.C. and V. T.: conception and design, manuscript writing, collection and/or assembly of data and final approval of manuscript; I.K.: conception and design, manuscript writing, collection and/or assembly of data, financial support and final approval of manuscript.

DISCLOSURE OF POTENTIAL CONFLICTS OF INTEREST

The authors indicate no potential conflicts of interest.

REFERENCES

1 Lozano R et al. Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: A systematic analysis for the Global Burden of Disease Study 2010. Lancet 2012;380:2095–2128. doi:10.1016/S0140-6736(12)61728-0

2 Mozaffarian D et al. Heart disease and stroke statistics–2015 update: A report from the American Heart Association. Circulation 2015;131:e29–e322. doi:10.1161/CIR.0000000000000152

3 Bergmann O et al. Evidence for cardiomyocyte renewal in humans. Science 2009;324:98–102. doi:10.1126/science.1164680

4 McMurray JJ et al. ESC guidelines for the diagnosis and treatment of acute and chronic heart failure 2012: The Task Force for the Diagnosis and Treatment of Acute and Chronic Heart Failure 2012 of the European Society of Cardiology. Developed in collaboration with the Heart Failure Association (HFA) of the ESC. Eur J Heart Failure 2012;14:803–869. doi:10.1093/eurjhf/hfs105

5 Yancy CW et al. 2013 ACCF/AHA guideline for the management of heart failure: Executive summary: A report of the American College of Cardiology Foundation/American Heart Association Task Force on practice guidelines. Circulation 2013;128:1810–1852. doi:10.1161/CIR.0b013e31829e8807

6 Wollert KC, Meyer GP, Lotz J et al. Intracoronary autologous bone-marrow cell transfer after myocardial infarction: The BOOST randomised controlled clinical trial. Lancet 2004;364:141–148. doi:10.1016/S0140-6736(04)16626-9

7 Janssens S, Dubois C, Bogaert J et al. Autologous bone marrow-derived stem-cell transfer in patients with ST-segment elevation myocardial infarction: Double-blind, randomised controlled trial. Lancet 2006;367:113–121. doi:10.1016/S0140-6736(05)67861-0

8 Lunde K, Solheim S, Aakhus S et al. Intracoronary injection of mononuclear bone marrow cells in acute myocardial infarction. New Engl J Med 2006;355:1199–1209. doi:10.1056/NEJMoa055706

9 Schachinger V, Erbs S, Elsasser A et al. Intracoronary bone marrow-derived progenitor cells in acute myocardial infarction. New Engl J Med 2006;355:1210–1221. doi:10.1056/NEJMoa060186

10 Huikuri HV, Kervinen K, Niemela M et al. Effects of intracoronary injection of mononuclear bone marrow cells on left ventricular function, arrhythmia risk profile, and restenosis after thrombolytic therapy of acute myocardial infarction. Eur Heart J 2008;29:2723–2732. doi:10.1093/eurheartj/ehn436

11 Choudry F, Hamshere S, Saunders N et al. A randomized double-blind control study of early intra-coronary autologous bone marrow cell infusion in acute myocardial infarction: The REGENERATE-AMI clinical trialdagger. Eur Heart J 2016;37:256–263. doi:10.1093/eurheartj/ehv493

12 Traverse JH, Henry TD, Pepine CJ et al. Effect of the use and timing of bone marrow mononuclear cell delivery on left ventricular function after acute myocardial infarction: The TIME randomized trial. JAMA 2012;308:2380–2389. doi:10.1001/jama.2012.28726

13 Traverse JH, Henry TD, Vaughan DE, et al. LateTIME: A phase-II, randomized, double-blinded, placebo-controlled, pilot trial evaluating the safety and effect of administration of bone marrow mononuclear cells 2 to 3 weeks after acute myocardial infarction. Tex Heart Inst J 2010;37:412–420.

14 Perin EC, Willerson JT, Pepine CJ et al. Effect of transendocardial delivery of autologous bone marrow mononuclear cells on functional capacity, left ventricular function, and perfusion in chronic heart failure: The FOCUS-CCTRN trial. JAMA 2012;307:1717–1726. doi:10.1001/jama.2012.418

15 Assmus B, Honold J, Schachinger V et al. Transcoronary transplantation of progenitor cells after myocardial infarction. New Engl J Med 2006;355:1222–1232. doi:10.1056/NEJMoa051779

16 Henry TD, Traverse JH, Hammon BL et al. Safety and efficacy of ixmyelocel-T: An expanded, autologous multi-cellular therapy, in dilated cardiomyopathy. Circ Res 2014;115:730–737. doi:10.1161/CIRCRESAHA.115.304554

17 Patel AN, Henry TD, Quyyumi AA et al. Ixmyelocel-T for patients with ischaemic heart failure: A prospective randomised double-blind trial. Lancet 2016;387:2412–2421. doi:10.1016/S0140-6736(16)30137-4

18 Bartunek J, Behfar A, Dolatabadi D et al. Cardiopoietic stem cell therapy in heart failure: The C-CURE (Cardiopoietic stem Cell therapy in heart failURE) multicenter randomized trial with lineage-specified biologics. J Am Coll Cardiol 2013;61:2329–2338. doi:10.1016/j.jacc.2013.02.071

19 Bartunek J, Davison B, Sherman W et al. Congestive Heart Failure Cardiopoietic Regenerative Therapy (CHART-1) trial design. Eur J Heart Fail 2016;18:160–168. doi:10.1002/ejhf.434

20 Perin EC, Borow KM, Silva GV et al. A phase II dose-escalation study of allogeneic mesenchymal precursor cells in patients with ischemic or nonischemic heart failure. Circ Res 2015;117:576–584. doi:10.1161/CIRCRESAHA.115.306332

21 Hare JM, Fishman JE, Gerstenblith G et al. Comparison of allogeneic vs autologous bone marrow-derived mesenchymal stem cells delivered by transendocardial injection in patients with ischemic cardiomyopathy: The POSEIDON randomized trial. Jama 2012;308:2369–2379. doi:10.1001/jama.2012.25321

22 Bolli R, Chugh AR, D'Amario D et al. Cardiac stem cells in patients with ischaemic cardiomyopathy (SCIPIO): Initial results of a randomised phase 1 trial. Lancet 2011;378:1847–1857. doi:10.1016/S0140-6736(11)61590-0

23 Chugh AR, Beache GM, Loughran JH et al. Administration of cardiac stem cells in patients with ischemic cardiomyopathy: The SCIPIO trial: Surgical aspects and interim analysis of myocardial function and viability by magnetic resonance. Circulation 2012;126:S54–S64. doi:10.1161/CIRCULATIONAHA.112.092627

24 Makkar RR, Smith RR, Cheng K et al. Intracoronary cardiosphere-derived cells for heart regeneration after myocardial infarction (CADUCEUS): A prospective, randomised phase 1 trial. Lancet 2012;379:895–904. doi:10.1016/S0140-6736(12)60195-0

25 Malliaras K, Makkar RR, Smith RR et al. Intracoronary cardiosphere-derived cells after myocardial infarction: Evidence of therapeutic regeneration in the final 1-year results of the CADUCEUS trial (CArdiosphere-Derived aUtologous stem CElls to reverse ventricUlar dySfunction). J Am Coll Cardiol 2014;63:110–122. doi:10.1016/j.jacc.2013.08.724

26 Matsubara H. First-in-man Cell Therapy Clinical Trial for Heart Failure—AutoLogous human Cardic-Derived Stem Cell to Treat Ischemic cardiomyopathy (ALCADIA). J Card Fail 2011;17:S130. doi:10.1016/j.cardfail06.408.

27 Menasche P, Vanneaux V, Hagege A et al. Human embryonic stem cell-derived cardiac progenitors for severe heart failure treatment: First clinical case report. Eur Heart J 2015;36:2011–2017. doi:10.1093/eurheartj/ehv189

28 Pittenger MF, Mackay AM, Beck SC et al. Multilineage potential of adult human mesenchymal stem cells. Science 1999;284:143–147.

29 Hamano K, Nishida M, Hirata K et al. Local implantation of autologous bone marrow cells for therapeutic angiogenesis in patients with ischemic heart disease: Clinical trial and preliminary results. Japan Circ J 2001;65:845–847.

30 Behfar A, Terzic A. Derivation of a cardiopoietic population from human mesenchymal stem cells yields cardiac progeny. Nat Clin Pract Cardiovasc Med 2006;3Suppl 1:S78–S82. doi:10.1038/ncpcardio0429

31 Mielewczik M, Cole GD, Nowbar AN et al. The C-CURE Randomized Clinical Trial (Cardiopoietic stem Cell therapy in heart failURE). J Am Coll Cardiol 2013;62:2453. doi:10.1016/j.jacc.2013.09.013

32 Ledford KJ, Murphy N, Zeigler F et al. Therapeutic potential of ixmyelocel-T, an expanded autologous multicellular therapy for treatment of ischemic cardiovascular diseases. Stem Cell Res Ther 2015;6:25. doi:10.1186/s13287-015-0007-3

33 Ledford KJ, Zeigler F, Bartel RL. Ixmyelocel-T, an expanded multicellular therapy, contains a unique population of M2-like macrophages. Stem Cell Res Ther 2013;4:134. doi:10.1186/scrt345

34 Karantalis V, Schulman IH, Balkan W et al. Allogeneic cell therapy: A new paradigm in therapeutics. Circ Res 2015;116:12–15. doi:10.1161/CIRCRESAHA.114.305495

35 Fisher SA, Doree C, Mathur A et al. Meta-analysis of cell therapy trials for patients with heart failure. Circ Res 2015;116:1361–1377. doi:10.1161/CIRCRESAHA.116.304386

36 Afzal MR, Samanta A, Shah ZI, et al. Adult bone marrow cell therapy for ischemic heart disease: Evidence and insights from randomized controlled trials. Circ Res 2015;117:558–575. doi:10.1161/CIRCRESAHA.114.304792

37 Gyongyosi M, Wojakowski W, Navarese EP et al. Meta-analyses of human cell-based cardiac regeneration therapies: Controversies in

meta-analyses results on cardiac cell-based regenerative studies. Circ Res 2016;118:1254–1263. doi:10.1161/CIRCRESAHA.115.307347

38 Nowbar AN, Mielewczik M, Karavassilis M et al. Discrepancies in autologous bone marrow stem cell trials and enhancement of ejection fraction (DAMASCENE): Weighted regression and meta-analysis. Bmj 2014;348: g2688. doi:10.1136/bmj.g2688

39 Beltrami AP et al. Adult cardiac stem cells are multipotent and support myocardial regeneration. Cell 2003;114:763–776.

40 Koudstaal S, Jansen Of Lorkeers SJ, Gaetani R et al. Concise review: Heart regeneration and the role of cardiac stem cells. Stem Cells Transl Med 2013;2:434–443. doi:10.5966/sctm.2013-0001

41 Keith MC, Bolli R. String theory" of c-kit(-pos) cardiac cells: A new paradigm regarding the nature of these cells that may reconcile apparently discrepant results. Circ Res 2015; 116:1216–1230. doi:10.1161/CIRCRESAHA.116.305557

42 The Lancet Editors. Expression of concern: The SCIPIO trial. Lancet 2014;383:1279. doi:10.1016/S0140-6736(14)60608-5

43 Smith RR, Barile L, Cho HC et al. Regenerative potential of cardiosphere-derived cells expanded from percutaneous endomyocardial biopsy specimens. Circulation 2007;115:896–908. doi:10.1161/CIRCULATIONAHA.106.655209

44 Li TS, Cheng K, Lee ST et al. Cardiospheres recapitulate a niche-like microenvironment rich in stemness and cell-matrix interactions, rationalizing their enhanced functional potency for myocardial repair. Stem Cells 2010;28:2088–2098. doi:10.1002/stem.532

45 Takehara N, Tsutsumi Y, Tateishi K et al. Controlled delivery of basic fibroblast growth factor promotes human cardiosphere-derived cell engraftment to enhance cardiac repair for chronic myocardial infarction. J Am Coll Cardiol 2008;52:1858–1865. doi:10.1016/j.jacc.2008.06.052

46 Freund C, Mummery CL. Prospects for pluripotent stem cell-derived cardiomyocytes in cardiac cell therapy and as disease models. J Cell Biochem 2009;107:592–599. doi:10.1002/jcb.22164

47 Burridge PW, Keller G, Gold JD et al. Production of de novo cardiomyocytes: Human pluripotent stem cell differentiation and direct reprogramming. Cell Stem Cell 2012;10:16–28. doi:10.1016/j.stem.2011.12.013

48 Gerbin KA, Murry CE. The winding road to regenerating the human heart. Cardiovasc Pathol 2015;24:133–140. doi:10.1016/j.carpath.2015.02.004

49 Simonson OE, Domogatskaya A, Volchkov P et al. The safety of human pluripotent stem cells in clinical treatment. Ann Med 2015;47:370–380. doi:10.3109/07853890.2015.1051579

50 Karakikes I, Ameen M, Termglinchan V et al. Human induced pluripotent stem cell-derived cardiomyocytes: Insights into molecular, cellular, and functional phenotypes. Circ Res 2015;117:80–88. doi:10.1161/CIRCRESAHA.117.305365

51 Braunwald E. Research advances in heart failure: A compendium. Circ Res 2013;113:633–645. doi:10.1161/CIRCRESAHA.113.302254

52 Hedman M, Hartikainen J, Syvanne M et al. Safety and feasibility of catheter-based local intracoronary vascular endothelial growth factor gene transfer in the prevention of post-angioplasty and in-stent restenosis and in the treatment of chronic myocardial ischemia: Phase II results of the Kuopio Angiogenesis Trial (KAT). Circulation 2003;107:2677–2683. doi:10.1161/01.CIR.0000070540.80780.92

53 Henry TD, Annex BH, McKendall GR et al. The VIVA trial: Vascular endothelial growth factor in Ischemia for Vascular Angiogenesis. Circulation 2003;107:1359–1365.

54 Hassinen I, Hartikainen J, Hedman A et al. Abstract 11987: Adenoviral intramyocardial VEGF-D gene transfer increases myocardial perfusion in refractory angina patients. Circulation 2015;132:A11987–A11987.

55 Kastrup J, Jorgensen E, Ruck A et al. Direct intramyocardial plasmid vascular endothelial growth factor-A165 gene therapy in patients with stable severe angina pectoris A randomized double-blind placebo-controlled study: The Euroinject One trial. J Am Coll Cardiol 2005;45:982–988. doi:10.1016/j.jacc.2004.12.068

56 Stewart DJ, Kutryk MJ, Fitchett D et al. VEGF gene therapy fails to improve perfusion of ischemic myocardium in patients with advanced coronary disease: Results of the NORTHERN trial. Mol Ther 2009;17:1109–1115. doi:10.1038/mt.2009.70

57 Stewart DJ, Hilton JD, Arnold JM et al. Angiogenic gene therapy in patients with nonrevascularizable ischemic heart disease: A phase 2 randomized, controlled trial of AdVEGF(121) (AdVEGF121) versus maximum medical treatment. Gene Ther 2006;13:1503–1511. doi:10.1038/sj.gt.3302802

58 Henry TD, Grines CL, Watkins MW et al. Effects of Ad5FGF-4 in patients with angina: An analysis of pooled data from the AGENT-3 and AGENT-4 trials. J Am Coll Cardiol 2007; 50:1038–1046. doi:10.1016/j.jacc.2007.06.010

59 Grines CL, Watkins MW, Helmer G et al. Angiogenic Gene Therapy (AGENT) trial in patients with stable angina pectoris. Circulation 2002;105:1291–1297.

60 Grines CL, Watkins MW, Mahmarian JJ et al. A randomized, double-blind, placebo-controlled trial of Ad5FGF-4 gene therapy and its effect on myocardial perfusion in patients with stable angina. J Am Coll Cardiol 2003;42:1339–1347.

61 Hammond H, Penny WF, Traverse JH et al. Intracoronary gene transfer of adenylyl cyclase 6 in patients with heart failure: A randomized clinical trial. JAMA Cardiol 2016;1:163–171. doi:10.1001/jamacardio.2016.0008

62 Hajjar RJ, Zsebo K, Deckelbaum L et al. Design of a phase 1/2 trial of intracoronary administration of AAV1/SERCA2a in patients with heart failure. J Card Fail 2008;14:355–367. doi:10.1016/j.cardfail.2008.02.005

63 Jaski BE, Jessup ML, Mancini DM et al. Calcium upregulation by percutaneous administration of gene therapy in cardiac disease (CUPID Trial), a first-in-human phase 1/2 clinical tria. J Card Fail 2009;15:171–181. l. doi:10.1016/j.cardfail.2009.01.013

64 Zsebo K, Yaroshinsky A, Rudy JJ et al. Long-term effects of AAV1/SERCA2a gene transfer in patients with severe heart failure: Analysis of recurrent cardiovascular events and mortality. Circ Res 2014;114:101–108. doi:10.1161/CIRCRESAHA.113.302421

65 Jessup M, Greenberg B, Mancini D et al. Calcium Upregulation by Percutaneous Administration of Gene Therapy in Cardiac Disease (CUPID): A phase 2 trial of intracoronary gene therapy of sarcoplasmic reticulum Ca2+-ATPase in patients with advanced heart failure. Circulation 2011;124:304–313. doi:10.1161/CIRCULATIONAHA.111.022889

66 Greenberg B, Butler J, Felker GM et al. Calcium upregulation by percutaneous administration of gene therapy in patients with cardiac disease (CUPID 2): A randomised, multinational, double-blind, placebo-controlled, phase 2b trial. Lancet doi:10.1016/S0140-6736(16)00082-9 2016;

67 Penn MS, Mendelsohn FO, Schaer GL et al. An open-label dose escalation study to evaluate the safety of administration of non-viral stromal cell-derived factor-1 plasmid to treat symptomatic ischemic heart failure. Circ Res 2013;112:816–825. doi:10.1161/CIRCRESAHA.111.300440

68 Chung ES, Miller L, Patel AN et al. Changes in ventricular remodelling and clinical status during the year following a single administration of stromal cell-derived factor-1 non-viral gene therapy in chronic ischaemic heart failure patients: The STOP-HF randomized Phase II trial. Eur Heart J 2015;36:2228–2238. doi:10.1093/eurheartj/ehv254

69 Lompre AM, Hajjar RJ, Harding SE et al. Ca2+ cycling and new therapeutic approaches for heart failure. Circulation 2010; 121:822–830. doi:10.1161/CIRCULATIONAHA.109.890954

70 Kranias EG, Hajjar RJ. Modulation of cardiac contractility by the phospholamban/SERCA2a regulatome. Circ Res 2012;110:1646–1660. doi:10.1161/CIRCRESAHA.111.259754

71 Ritterhoff J, Most P. Targeting S100A1 in heart failure. Gene Ther 2012;19:613–621. doi:10.1038/gt.2012.8

72 Feldman AM. Adenylyl cyclase: A new target for heart failure therapeutics. Circulation 2002;105:1876–1878.

73 Tang T, Gao MH, Roth DM et al. Adenylyl cyclase type VI corrects cardiac sarcoplasmic reticulum calcium uptake defects in cardiomyopathy. Am J Physiol Heart Circ Physiol 2004;287:H1906–H1912. doi:10.1152/ajpheart.00356.2004

74 Rebolledo B, Lai NC, Gao MH et al. Adenylylcyclase gene transfer increases function of the failing heart. Hum Gene Ther 2006;17:1043–1048. doi:10.1089/hum.2006.17.1043

75 Askari AT, Unzek S, Popovic ZB et al. Effect of stromal-cell-derived factor 1 on stem-cell homing and tissue regeneration in ischaemic cardiomyopathy. Lancet 2003;362:697–703. doi:10.1016/S0140-6736(03)14232-8

76 Yamaguchi J, Kusano KF, Masuo O et al. Stromal cell-derived factor-1 effects on ex vivo expanded endothelial progenitor cell recruitment for ischemic neovascularization. Circulation 2003;107:1322–1328.

77 Ali SR, Hippenmeyer S, Saadat LV et al. Existing cardiomyocytes generate cardiomyocytes at a low rate after birth in mice. Proc Natl Acad Sci USA 2014;111:8850–8855. doi:10.1073/pnas.1408233111

78 Senyo SE, Steinhauser ML, Pizzimenti CL et al. Mammalian heart renewal by pre-existing cardiomyocytes. Nature 2013;493:433–436. doi:10.1038/nature11682

79 Kimura W, Xiao F, Canseco DC, et al. Hypoxia fate mapping identifies cycling cardiomyocytes in the adult heart. Nature 2015;523:226–230. doi:10.1038/nature14582

80 Nakada Y, Canseco DC, Thet S et al. Hypoxia induces heart regeneration in adult mice. Nature doi:10.1038/nature20173 2016;

81 Kim H, Kim JS. A guide to genome engineering with programmable nucleases. Nat Rev Genet 2014;15:321–334. doi:10.1038/nrg3686

82 Doudna JA, Charpentier E. Genome editing. The new frontier of genome engineering with CRISPR-Cas9. Science 2014;346:1258096. doi:10.1126/science.1258096

83 Nelson CE, Hakim CH, Ousterout DG et al. In vivo genome editing improves muscle function in a mouse model of Duchenne muscular dystrophy. Science 2016;351:403–407. doi:10.1126/science.aad5143

84 Tabebordbar M, Zhu K, Cheng JK et al. In vivo gene editing in dystrophic mouse muscle and muscle stem cells. Science 2016;351:407–411. doi:10.1126/science.aad5177

85 Long C, Amoasii L, Mireault AA et al. Postnatal genome editing partially restores dystrophin expression in a mouse model of muscular dystrophy. Science 2016;351:400–403. doi:10.1126/science.aad5725

86 Karakikes I, Stillitano F, Nonnenmacher M et al. Correction of human phospholamban R14del mutation associated with cardiomyopathy using targeted nucleases and combination therapy. Nat Commun 2015;6:6955. doi:10.1038/ncomms7955