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CONGESTIVE HEART FAILURE (J LINDENFELD, SECTION EDITOR)

Gene Therapy for Heart Failure: Where Do We Stand?

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Abstract Advances in understanding of the molecular basis of myocardial dysfunction, together with the development of increasingly efficient gene transfer technology, has placed heart failure within reach of gene-based therapy. Multiple components of cardiac contractility, including the Beta-adrenergic system, the calcium channel cycling pathway, and cytokine mediated cell proliferation, have been identified as appropriate targets for gene therapy. The development of efficient and safe vectors such as adeno-associated viruses and polymer nanoparticles has provided an opportunity for clinical application for gene therapy. The recent successful and safe completion of a phase 2 trial targeting the sarcoplasmic reticulum calcium ATPase pump (SERCA2a) has the potential to open a new era for gene therapy in the treatment of heart failure.

Keywords Heart failure · Gene therapy · Cardiac gene transfer · Sarcoplasmic reticulum · SERCA2a · Calcium handling · Phospholamban · Adeno-associated vectors

Introduction

The treatment of cardiovascular disease (CVD) has advanced significantly with greater understanding of the molecular pathophysiology in coronary artery disease and heart failure. Cell surface receptor modulators, β-blockers and angiotensin receptor antagonists have proven to be pharmacologically viable targets in small molecule design for treating cardiovascular disease. While progress in conventional treatment modalities is making steady and incremental gains to reduce CVD burden, 20 million Americans are diagnosed with CVD, and there remains an urgent need to explore new therapeutic approaches.

Gene therapy was initially envisioned as a treatment strategy for inherited monogenic disorders. It is now apparent that gene therapy has broader potentials including acquired polygenic diseases such as peripheral vascular disease, ischemic heart disease, arrhythmias, and congestive heart failure. Advances in the understanding of the molecular basis of cardiovascular disease, together, with the evolution of increasingly safe and efficient gene transfer technologies, has placed cardiovascular disease within reach of gene-based therapies.

This review will focus on both targets of gene therapy in cardiovascular disease, as well as advancements in vector design and application in context of clinical trials in heart failure. The current cardiologist will benefit from further understanding of the techniques and rationale of cardiovascular gene therapy as this therapeutic strategy enters the clinical realm.

Molecular Targets

The last 20 years witnessed significant evolution in our understanding of the pathophysiology of heart failure in its molecular and cellular dimensions. We will discuss some of the most important systems targeted to restore the function of failing cardiomyocytes.

The β-adrenergic System

The myocardial remodeling process associated with heart failure is known to alter β-adrenergic signaling. The increased ionotropic requirements associated with contractile

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dysfunction cause downregulation of B-adrenergic receptors (β-AR) in the process of sympathetic desensitization. Overexpression of β-AR was initially tested as a simple way to overcome β-AR downregulation. Transgenic mice overexpressing the human β1-ARs suffered from severe cardiomyopathy [1]. In contrast, mice with cardiac overexpression of β2-AR demonstrated increased basal myocardial adenylyl cyclase activity with increased left ventricular function [2]. Both direct and intracoronary myocardial delivery of Adenovirus containing the human β2-AR transgene has resulted in enhanced cardiac performance in rodents and mammalian models [3, 4].

Downstream activators were also explored as potential targets for adrenergic regulation. Agonist-dependent desensitization is mediated by a family of G protein-coupled receptor kinases (GRKs) which phosphorylate the agonist-occupied receptors resulting in functional uncoupling. GRK2 is the most expressed GRK in the heart. It has been implicated in the pathogenesis of dysfunctional cardiac β-AR signaling accounting for a deleterious activity in the failing heart [5]. Studies in mice in which HF was induced by a myocardial infarction, showed that selective GRK2 ablation 10 days postinfarction resulted in increased survival, halted ventricular remodeling, and enhanced cardiac contractile performance [6]. A peptide termed βARKct capable of inhibiting GRK2 mediated β-AR desensitization has been evaluated in vivo in animals. Using intracoronary adenovirus-mediated βARKct transgene delivery to rabbits 3 weeks after an induced myocardial infarction demonstrated a marked reversal of ventricular dysfunction [7]. More recent studies have focused on overexpressing βARKct in large animal models [8].

Altering other components of the G-protein mediated system including Adenylyl Cyclase type VI (AC VI), seem to have a favorable response in animal models. Overexpression of AC VI in transgenic mice resulted in improved cardiac function in response to adrenergic stimulation along with increased cAMP production in isolated cardiac myocytes. Importantly, AC VI had a neutral effect on basal heart function and was not associated with any structural heart abnormalities [9]. In a pacing model of HF in pigs, intracoronary delivery of adenovirus encoding AC VI resulted in improved LV function and remodeling, associated with increased cAMP generating capacity [10]. The favorable effects of AC VI in preclinical studies are encouraging and this approach is currently under investigation for initiation of clinical trials in patients with HF [11].

Calcium Cycling Proteins

More than 20 years ago, Gwathmey et al. first reported that calcium cycling is abnormal in human heart failure [12]. Further examination found that the sarcoplasmic calcium channel SERCA2a, which pumps calcium into SR during diastole, was implicated in this process, with decreased SERCA2a activity regardless of the etiology of the heart failure [13–16]. Improvement in cardiac contractility after gene transfer of SERCA2a has been demonstrated in a large number of experimental models of heart failure [17, 18]. More importantly, long-term overexpression of SERCA2a by intracoronary delivery of AAV carrying SERCA2a has been associated with preserved systolic function and improved ventricular remodeling in a swine volume-overload model of HF [19]. Beyond their effects on enhancing contractility, SERCA2a gene transfer has been shown to restore the energetics state of the heart [20, 21] both in terms of energy supply and utilization, decrease ventricular arrhythmias [22•, 23–25], and enhance coronary flow through activation of endothelial nitric oxide synthase (eNOS) in endothelial cells [26•].

The importance of calcium channels in the etiology of heart failure led to the exploration of channel modifiers, one of which is the channel inhibitor Phospholamban (PLN). Decreasing PLN in human cardiac myocytes showed an improvement in contraction and relaxation velocities similar to the benefit seen with gene transfer of SERCA2a [27]. Silencing of PLN expression in a sheep HF model resulted in improved SERCA activity along with improved systolic and diastolic LV function [28]. An RNAi vector generated stable cardiac production of a regulatory RNA sequence, which in turn suppressed phospholamban expression. SERCA2a protein was subsequently increased accompanied by restoration of systolic and diastolic cardiac function [29].

Affectors of Phospholamban, including inhibitor PP1 and secondary activator I-1, were also found to be modified in HF. Phospholamban inhibitor PP1 is elevated in HF, resulting in dephosphorylation of PLN. Overexpression of PP1 or ablation of I-1 in murine hearts has been associated with decreased β-AR-mediated contractile responses, depressed cardiac function and premature death consistent with HF [30–32]. Expression of a constitutively active I-1 in transgenic mice led to PP1 inhibition with increased phosphorylation of PLN and improved cardiac contractility. A recent study on transgenic mice expressing active I-1 confirmed the relationship between phosphorylation of PLN and SERCA2a activity. I-1 expression ameliorated ischemia/reperfusion-induced injury by reducing the infarct size and improving contractile recovery in addition to decreasing biomarkers of apoptosis and ER stress response [30–32].

S100 is part of a family of Ca^{2+}-modulated proteins implicated in intracellular regulatory activities, with S100A1 enhancing the activity of both RYRs and SERCA2a [33]. In a rat model of HF, AAV6-mediated long term expression of S100A1 resulted in a sustained in vivo reversal of LV dysfunction and remodeling [34, 35]. More recently AAV9 gene

transfer of S100A1 in a pre-clinical model of ischemic cardiomyopathy induced dramatic improvements in contractile function reinforcing the rationale that a clinical trial of S100A1 gene therapy for human heart failure should be forthcoming.

Recently, Kho et al. reported that the levels and activity of SERCA2a in cardiomyocytes are modulated in parallel with the levels of a cytoplasmic protein, small ubiquitin-like modifier type 1 (SUMO1) [36•]. SUMOs are a family of peptides that alter the function of other proteins in cells through a post-translational modification described as sumoylation. Sumoylation is involved in the modulation of various intracellular processes. Kho et al. found that increasing SUMO1 levels in the pig model by AAV9 gene transfer led to a restoration of SERCA2a levels, improved hemodynamic performance, and reduced mortality among the animals with heart failure.

Other Targets

The stromal cell–derived factor 1/chemokine receptor 4 (SDF1/CXCR4) complex has emerged as a therapeutic target in ischemic heart failure [37] due to the ability of the SDF-1-CXCR4 system to promote the homing of stem cells to infarcted myocardium. A clinical trial is underway to investigate the therapeutic benefit of SDF-1 overexpression in ischemic cardiomyopathy [38]. In parallel, existing literature highlights the direct effects of CXCR4 on the myocardium and the cardiac myocyte. SDF-1 was shown to decrease myocardial contractility ex vivo in cardiac myocytes [39]. One recent report has shown increased ischemia-reperfusion injury in rat hearts overexpressing CXCR4 [40], while another report investigated the modulation of beta-adrenergic receptor signaling by SDF-1 and CXCR4 [41], raising interrogations over the potential complex interaction between these chemokines and the cardiovascular system.

Gene Therapy Vectors

The development of gene transfer technology requires a detailed understanding of the target cell and the transgene biology. Vector design must account for the temporal and spatial patterns of the specific cardiovascular pathophysiological process, whether they are global processes like heart failure or focal processes such as nodal dysfunction. For example, vector design in heart failure must account for persistent transgene expression to ensure long term improvement in 5-yr outcomes, as well as broad transfection to ensure significant impact on ventricular function. Each vector delivery system provides different specificities regarding these concerns, and an appropriate choice for a delivery system will guide success in therapy. Gene delivery systems can be classified into two categories, non-viral physicochemical systems and recombinant viral systems, with each having unique profiles in gene transfer expression. The most commonly used vector systems will be covered in the following discussion. In Table 1 we list and compare the different properties of the most commonly used vectors for cardiovascular research.

Non-Viral Vectors

The gene carrier in non-viral gene therapy constructs is a double stranded circular plasmid. These plasmids typically include a constitutive promoter sequence such as the Cytomegalovirus (CMV) or roux sarcoma promoters, as well as enhancer sequences that provide tissue specificity. There are several benefits to plasmid DNA vector based systems including ease of manufacturing, low cost and reduced risk of systemic immunological responses. Delivery of naked plasmids to tissue however, has not been shown to provide adequate transfection profiles. Rapid systemic degradation of plasmids, poor cellular entry, intracellular compartmentalization and transient expression of gene products all limit the utility of naked plasmids as a transfection tool.

Transient expression due to de novo methylation, histone modification and heterochromatin formation is being prevented through improved plasmid design. Recently identified matrix attachment region (MAR) elements have been shown to prevent transgene silencing in part by inhibiting DNA methylation leading to long-term expression in vivo [42–44]. It is hypothesized that MAR elements interact with the nuclear matrix effectively insulating the transgene and creating independent euchromatin domains preventing methylation and heterochromatin formation [42, 45]. Incorporation of MAR elements may be a useful strategy in prolonging transgene expression in non-viral DNA based gene transfer therapies.

Plasmid stability is being addressed through carrier molecules including liposomal and polymer systems. The use of liposome-DNA complexes provides stability of plasmid DNA in the systemic circulation; however, it is cleared quickly and mainly induces expression in the lungs. Furthermore, these liposomal complexes do not offer DNA escape mechanisms from the intracellular endosomal complex. Polymer based DNA complexes involving polyethylenimines (PEI) and poly(amido amine) complexes (PAA) have shown usefulness in improving gene transduction though enhanced uptake, reduced cytotoxicity and protection from endosomal degradation [46]. PAA carboxylic modifications have further improved cellular transduction and continued development of PAAs are a promising strategy for gene delivery mechanisms [47].

Table 1 Comparison of major vector systems

Vector	Non-viral	Adenoviral	AAV	Lentiviral
Diameter	N/A	60-90 nm	20-25 nm	100 nm
Genome	DNA	dsDNA	ssDNA	ssRNA
Insert capacity, kb	>50 kb	7-36 kb	4.8 kb	7-10 kb
Integration	No	No	No	Pseudo-random
Cell type	Dividing/non-dividing	Dividing/non-dividing	Dividing/non-dividing	Dividing/non-dividing
Peak cardiac expression	2-4 days	1-4 days	2-4 weeks	4-6 days
Pattern of transgene expression	Low - transient	High - transient	Moderate - long term	Moderate - long term
Tissue tropism	No	Liver, neuronal, striated muscles, respiratory	Heart for cardiotropic AAVs	$CD4^+$ cells
Pathogenicity	No	Common cold	No	HIV-related virus
Neutralizing antibodies	None	Present	Present in 20–80 % of population	Maybe
Immune response	Low inflammatory	Robust, cytotoxic and immunogenic	Minimal and transient	Minimal
Risk of insertional mutagenesis	None	Low	Rare	Present
Production	Easy	Easy	Moderate	Difficult
Genetic manipulation	Easy	Moderate	Difficult	Moderate
Clinical trial approved	Yes	Yes	Yes	No

AAV: Adeno-associated Viruses; CAR: Coxsackie–Adenovirus receptor; HSPG: Heparan sulfate proteoglycan; EGFR: Epidermal growth factor receptor

Viral Vectors

The predominant use of viral vectors in preclinical models of gene therapy and in human clinical trials is a reflection of the superior gene transfer efficiencies achievable with these systems. This efficiency is conferred as a result of utilizing virological elements securing favorable gene expression. The four most developed and clinically relevant viral vector systems in human clinical trials include retrovirus, lentivirus, adenovirus, and adeno-associated virus.

Retrovirus

Viral vectors from the family Retroviridae include retrovirus and lentivirus. Retroviruses contain single-stranded positive-sense RNA which contain a virally encoded reverse transcriptase. This generates double stranded DNA which is inserted into the host genome. The ability to insert genetic sequences directly into the genome provides an opportunity for long-term gene expression in transfected tissues. The use of retroviruses in cardiovascular gene therapy has been limited to research use however, for numerous reasons. One limitation is that Retroviruses require active mitosis for viral integration into the genome. Therefore, retroviruses are limited to infecting dividing cells and cannot efficiently transduce non-dividing cell types such as cardiomyocytes or quiescent endothelial or smooth muscle cells.

Another major limitation of retroviruses in clinical gene therapy is insertional mutagenesis [48]. Retroviral DNA integration is a pseudorandom process integrating primarily in promoter and enhancer regions of the host cell genome. In the trial of gene therapy for X-linked severe combined immunodeficiency (X-SCID) with Moloney based retroviruses, five out of a total of 20 patients developed T-cell acute lymphoblastic leukemia due to insertion of the viral genome near the T-cell proto-oncogene Lim-only 2 (LMO-2) promoter [49–51]. As a result there has been renewed interest in improving vector biosafety. Modifications such as the use self-inactivating vectors, the introduction of insulator sequences and targeting of genome integration sites are potential methods reducing the risk of insertional mutagenesis [49, 50, 52].

Lentivirus Vectors

Lentiviral vectors, also from the family Retroviridae, are ssRNA viruses utilizing reverse transcriptase and genome integration for long-term expression of transgenes. In contrast to retroviruses, lentiviral vectors are capable of transducing mitotically quiescent cells allowing for efficient transduction of cardiomyocytes and do not have the same predilection to activating proto-oncogenes. The LV pre-integration complex contains a nuclear localization signal allowing transport across the nuclear membrane via nuclear pore complexes of non-dividing cells [53]. 0The most

commonly used lentiviral vector system is based on the human immunodeficiency virus type 1 (HIV-1). Vector biosafety concerns associated with HIV-1 have led to the development of non-primate lentiviral vectors that do not induce seroconversion in humans. Several human clinical trials of lentiviral vectors have shown promise for various disorders including ADA-deficient SCID (Clinical Trial ID: NCT01380990) and HIV infection [54–56]. The safety and efficacy of these human clinical trials and the feature of long-term expression of transgenes in non-dividing cells is critical in further developing lentiviral based therapies for human cardiovascular disease. Furthermore, the random integration afforded by the lentivirus continues to be a concern.

Adenoviral Vectors

Adenovirus, from the family Adenoviridae, is a non-enveloped, non-integrating virus consisting of seven species (Adenovirus A-G) and 57 serotypes. Adenoviruses have large protein complexes that bind to CD46 or coxsackie-adenovirus receptor (CAR) depending on the serotype for viral binding and cellular entry. Upon cellular entry via clathrin-mediated endocytosis, the dsDNA is transported to the nucleus across nuclear pores, allowing efficient transduction of both mitotic and non-mitotic cells.

While providing high transfection efficiency, Adenoviral vectors provide only transient expression. Transgene expression levels peaks within 2–3 days, but return to undetectable levels by 2 weeks [57, 58]. Another disadvantage of Adv vectors are the presence of systemic neutralizing antibodies and the highly immunogenic potential of these viruses. Adenovirus elicits robust innate immune responses which can severely compromise organ function and patient health [59]. Third-generation gutless, or helper dependent, Adv have diminished immunogenic potential, however, significant risk remains. Finally, CAR receptors are located systemically, and are particularly prevalent in liver tissue, limiting adenoviral tissue specificity. Due to these limiting factors, adenoviral mediated strategies are declining in prevalence for cardiovascular gene therapy trials.

Adeno-Associated Virus (AAV) Vectors

Adeno-associated viruses are members of the family Parvoviridae and are non-enveloped, single-stranded DNA viruses. AAV are relatively small (20 nm) and therefore are limited in their genome capacity of 4.8 kb. AAV is not known to cause human pathology, infects both dividing and non-dividing cells, and elicits a reduced adaptive and innate immune response as compared to other viral vectors. There are 13 reported serotypes of AAV with varying degrees of tissue tropism depending on capsid protein structure. AAV1, 6, 8 and 9 have been identified as being the most cardiotropic, however, significant transduction in non-target tissues such as liver, skeletal muscle and lung persists. Various methods have improved AAV cardiotropism, with novel viral capsid AAV libraries being constructed though DNA shuffling and chimeric strain production. This strategy not only can be used to enhance tissue tropism but also can be utilized to produce AAV to evade naturally occurring neutralizing antibodies [60–64]. Neutralizing antibodies to various AAV serotypes are present in approximately 20-80 % of the population, therefore severely limiting potential therapeutic use of AAV and are a major exclusion criterion in many AAV-based clinical trials [65••]. Strategies such as directed evolution as well as chemical modification of capsid proteins by conjugation of polyethylene glycol may partially circumvent the presence of neutralizing antibodies.

The various vector systems all have different expression kinetics and tissue tropisms that must be taken into account when designing human gene therapy trials. In regards to cardiac gene therapy, α-Myosin heavy chain, myosin light chain kinase-2, and troponin T promoters have shown success in restricting transgene expression to the cardiac myocyte [66, 67]. In addition, the use of human brain natriuretic peptide promoters increases cardiac myocyte specificity and transgene regulation upon increased ventricular wall stress. Strategies utilizing hypoxia response elements and HIF1α promoters can be useful in restricting transgene expression of angiogenic factors during myocardial ischemia, such as acute MI and/or unstable angina [68, 69]. Finally, methods to temporally restrict transgene expression using ligand-inducible promoters may be suitable to turn on transgene expression when clinically necessary, including acute decompensated heart failure. This could be achieved through a tetracycline-on (Tet-on) or rapamycin-inducible constructs, which have both been shown to temporally regulate gene expression [70, 71].

Vector Delivery

The selection of an appropriate vector delivery method is critical for proper implementation of the therapeutic strategy and for efficient transgene expression in the myocardium. Vectors have unique profiles of bioavailability, transgene expression kinetics, and tissue tropisms; therefore it is vital to choose a vector delivery method that complements the vector as well as the disease process. Importantly, both the invasiveness of vector delivery method and the patient safety need critical assessment prior to initiating gene therapy trials for cardiovascular disease.

Coronary Artery and Venous Infusion

Percutaneous coronary artery catheterization is a minimally invasive and well-established procedure that allows homogenous gene delivery to each territory of the heart. The major advantages of this approach are that it is minimally invasive and relatively safe. However, the fast transit time through the vasculature limits vector residence in the myocardium [72]. An approach that focuses on enhancing the vector residence time in the coronary circulation is coronary venous blockade. Antegrade coronary infusion with a short occlusion of both a coronary artery and a coronary vein enhanced myocardial gene expression [73, 74]. Cardiac recirculation can also maximize vector exposure [28]. Kaye et al. engineered an extracorporal device that drains blood from the coronary sinus using an occlusion catheter and returns the oxygenated coronary venous blood to the left main coronary artery via a peristaltic pump (V-Focus, Osprey Medical Inc, St Paul, MN, USA) [75, 76]. In an ovine model of tachycardia-induced heart failure, the closed loop recirculation method was more efficient in the transduction of cardiomyocytes than antegrade coronary infusion, which also translated into a greater improvement of left ventricular function [28, 75]. In addition, this method allows the selective administration of endothelial permeabilizing agents without systemic side effects [77].

The coronary venous system provides an alternative route for percutaneous delivery in patients with arterial pathology, moreover; numerous studies have showed that retrograde coronary venous infusion is an alternative myocardial delivery method for cardioprotective drugs [78, 79]. Shortly thereafter, retrograde venous infusion was also explored as a method for gene transfer. The rationale for a high transduction efficiency is based on controlling the exposure the time of vector to the endothelium, and on increasing the pressure gradient of capillary filtration [80, 81].

Indeed, studies in large animal models demonstrated that an efficient and homogenous myocardial transduction can be achieved by retroinfusion into the coronary venous system [81–83]. Boeksteger et al. showed that gene expression after pressure-regulated retrograde venous infusion was significantly higher than after antegrade coronary delivery if the retroinfusion was accompanied by simultaneous induced ischemia [81]. Compared to percutaneous or surgical direct myocardial injection, retrograde venous infusion also achieved a more homogeneous and efficient reporter gene expression [82]. Similar to antegrade coronary artery infusion, a closed loop recirculation retrograde venous infusion approach is also feasible [84]. Recently, White et al. demonstrated an extremely high transduction efficiency in the majority of cardiomyocytes in sheep while minimizing collateral organ exposure using a retrograde recirculation method during cardio-pulmonary bypass (CPB) surgery [85]. This method is not without concerns including the risk of myocardial edema or hemorrhage, as well as technical challenges.

Direct Intramyocardial Injection

Intramyocardial injection is one of the most widely used gene transfer methods, ranging from small animal studies to clinical trials focusing on cardiac angiogenesis. The vectors are injected either epicardially or endocardially into the target area with a small gauge needle. The primary advantages of this method are that vector delivery bypasses the endothelial barrier and avoids interaction with neutralizing actors in the blood. Furthermore, there is minimal exposure of the vector to off target organs, although local administration cannot completely avoid some systemic vector distribution [86, 87].

The simplest approach, however invasive, is the injection during a thoracotomy [88]. Surgical delivery offers direct visual confirmation, which allows precise control of the injection site, including an infarct border. The endocardial approach requires a catheter with a retractable injection needle, and imaging guidance modality for determining the injection site [87]. This includes electrical mapping systems [89], fluoroscopy [90], echocardiography [91], and magnetic resonance imaging [92]. The application of this method for heart failure, where cardiomyocytes are globally impaired, might be limited by a circumscribed target area and inhomogeneous expression profiles [82, 88].

Pericardial Delivery

The pericardial space faces most of the cardiac wall except the septum. In heart failure, where widespread cardiac gene transfer with little systemic distribution is desired, this large-scale interface combined with the concept of a closed compartment can be a major advantage. Intrapericardial delivery is performed surgically in rodents [93], whereas for larger animals a percutaneous approach is available as well. The percutaneous access to the pericardial space can be achieved minimally invasive via a substernal/xiphoidal or transatrial puncture. It has been proven to be feasible and safe when guided by imaging techniques like fluoroscopy and intravascular ultrasound [94]. Myocardial access is a concern however, with tightly joined pericardial cells restricting transfection only to superficial myocardial layers [93–95]. This limitation can be partly overcome by the co-administration of various pharmacological agents. Proteolytic enzymes and polyethyleneimine have been shown to increase the penetration depth of the vectors and to allow progressive release, often at the cost of cardiac toxicity [93, 96]. Although vectors are injected into a closed space, some studies reported extra-cardiac gene

expression, probably due to the rapid turnover of the pericardium fluid through the lymphatic absorption [97]. A platform, which allows slow vector release together with a permeabilizing, non-toxic agent, may increase the potential of pericardial injection for gene delivery [94].

Clinical Trials

The first clinical trial of gene therapy in patients with HF was launched in the United States in 2007 [65••, 98]. Calcium Up-Regulation by Percutaneous Administration of Gene Therapy in Cardiac Disease (CUPID) is a multicenter trial designed to evaluate the safety profile and the biological effects of gene transfer of the SERCA2a cDNA by delivering a recombinant AAV1 (AAV1.SERCA2a) in patients with advanced HF. Participants in this trial were administered a single intracoronary infusion of AAV1.SERCA2a in an open-label approach [65••, 98]. Cohorts 1–4, of 3 patients each, received sequentially a single escalating dose of AAV1.SERCA2a. Twelve months follow-up of these patients showed an acceptable safety profile and improvements along multiple parameters [65••, 98].

In the phase 2 trial, 39 patients with advanced HF were randomized to receive intracoronary adeno-associated virus 1 (AAV1) mediated SERCA2a gene delivery in one of 3 doses versus placebo; low dose - 6×10^{11} DRP, middle dose - 3×10^{12} DRP, and high dose - 1×10^{13} DRP. Patient's symptoms (NYHA class, Minnesota Living With Heart Failure Questionnaire [MLWHFQ]), functional status (6 minute walk test [6MWT] and VO2 max), NT-proBNP levels, and echocardiographic measures were evaluated over 6 months. AAV1.SERCA2a treated patients demonstrated improvement or stabilization in both the quantitative and qualitative factors mentioned above versus placebo. Significant increases in time to adjudicated CV events, and a decreased frequency of CV events per patient were also observed in all patients receiving AAV1.SERCA2a in the first six months. Compared to placebo, no increases in adverse events, disease-related events, laboratory abnormalities, or arrhythmias were observed [99••].

Two other clinical trials targeting SERCA2a are currently enrolling patients. The first trial is in patients with advanced heart failure having received left ventricular assist devices at least one month prior to treatment and who will receive either AAV6.SERCA2a or saline. This trial is being conducted in the United Kingdom. A second phase 2 monocenter double blind randomized, placebo-controlled, parallel study will be held in the Institute of Cardiology Pitié-Salpêtrière, Paris, France with the primary objective to investigate the impact of AAV1.SERCA2a on cardiac remodeling parameters in patients with severe heart failure.

Numerous clinical trials involving other targets are also ongoing. Adenovirus-5 encoding human adenylyl cyclase type 6 is being delivered through intracoronary injection to patients with congestive heart failure. An additional trial is examining the effects of injecting SDF-1 directly into the myocardium of patients with ischemic heart disease [38].

Conclusions

The continued elucidation of the molecular mechanisms of heart failure, along with the development of cardiotropic gene vectors, have established gene therapy as a viable adjunctive treatment to mechanical and pharmacological therapies for heart failure. In the coming years, more targets will emerge that are amenable to genetic manipulations along with more advanced vector systems which will undoubtedly lead to safer and more effective clinical trials in gene therapy for heart failure.

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