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Sarcoplasmic reticulum Ca^{2+} ATPase as a therapeutic target for heart failure

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Abstract

Background—The cardiac isoform of the sarco/endoplasmic reticulum Ca^{2+} ATPase (SERCA2a) plays a major role in controlling excitation/contraction coupling. In both experimental and clinical heart failure (HF), SERCA2a expression is significantly reduced which leads to abnormal Ca^{2+} handling and deficient contractility. A large number of studies in isolated cardiac myocytes, in small and large animal models of HF showed that restoring SERCA2a expression by gene transfer corrects the contractile abnormalities and improves energetics and electrical remodeling. Following a long line of investigation, a clinical trial is underway to restore SERCA2a expression in patients with HF using adeno-associated virus type 1.

Objective—This review addresses the following issues regarding HF gene therapy: 1) new insights on calcium regulation by SERCA2a; 2) SERCA2a as a gene therapy target in animal models of HF; 3) advances in the development of viral vectors and gene delivery; 4) clinical trials of HF using SERCA2a

Methods—This review focuses on the new advances in SERCA2a- targeted gene therapy made in the last three years.

Conclusion—SERCA2a is an important therapeutic target in various cardiovascular disorders. Ongoing clinical gene therapy trials will provide answers on its safety and applicability.

Keywords

Sarcoplasmic reticulum Ca^{2+} ATPase; heart failure; gene therapy

Introduction

Heart failure (HF) remains a major cause of morbidity and mortality in the developed world. In the population under the age of 65 HF prevalence approaches 1%¹. Despite the fact that pharmacologic and device-based treatments have improved survival, the 5-year mortality rate remains at an alarming 50% in this population². Faced with these challenges, researchers have vigorously explored novel therapeutic options, including gene-based treatments. Over the last decade, important progress has been made in understanding the various intracellular and molecular mechanisms that become abnormal during HF^{3–5}. One of the key abnormalities in both human HF and experimental models of HF is abnormal intracellular calcium ion (Ca^{2+}) handling caused by a defect in sarcoplasmic reticulum (SR) function^{4, 6}. Deficient SR Ca^{2+} uptake in myocytes is associated with a decrease in the expression and activity of the sarco/ endoplasmic reticulum calcium ATPase cardiac isoform

2a, (SERCA2a)^{3–5}. Unloaded ($[Ca^{2+}]_{SR}$) in turn regulates the effectors of SR Ca^{2+} release and Ca^{2+} influx channels, further worsening the abnormal Ca^{2+} distribution. SERCAs play a critical role in the control of spatio-temporal patterns of intracellular calcium signaling – a pattern, that controls virtually every cellular function, including contraction, proliferation/hypertrophic growth and apoptosis^{6, 7}. Normalization of SERCA2a function has been shown to increase contractility in failing human cardiomyocytes and to improve hemodynamics along with survival in rodent and large animal models of HF^{3–5}. The overexpression of SERCA2a also has been found to restore energetic supply and to decrease ventricular arrhythmias in a model of ischemia/reperfusion^{8–10}. Furthermore, a number of novel beneficial mechanisms have been uncovered in vascular smooth muscle cells (VSMCs) and endothelial cells (EC)^{11, 12}. Therefore, SERCA2a is one of the most promising targets for the treatment of HF.

Advances in the understanding of the molecular basis of myocardial dysfunction together with the evolution of gene transfer technology, has finally placed CHF within reach of gene-based therapy^{5, 13, 14}. Myocardial dysfunction results in myocardium that has a mixture of diseased, dysfunctional and healthy myocytes. The goal of gene therapy is to treat dysfunctional myocytes and to prevent disease or loss of healthy myocytes.

This review focuses on gene therapy using SERCA2a or small molecules regulating SERCA2a activity to treat HF. We present new insight in the mechanism of Ca^{2+} regulation by SERCA2a, examine the complex beneficial effects as well as the risk associated with SERCA2a overexpression. We describe the new advances in the development of viral vectors and gene delivery and discuss the potential of SERCA2a as a therapeutic target in cardiovascular disease.

1. Calcium cycling abnormalities in heart failure

Calcium is crucial to the very process that enables the chambers of the heart to contract and relax, a process called excitation-contraction coupling^{4–6}. During systole, the action potential induces a minor Ca^{2+} -influx through sarcolemmal L-type calcium channel (LTCC) (Figure 1). This triggers further calcium release from the SR via the major SR Ca^{2+} -release channel ryanodine receptor (RyR2, the cardiac isoform of RyR). The combination of Ca^{2+} influx and release raises free intracellular Ca^{2+} concentration ($[Ca^{2+}]$) from 0.1–0.2 μ M to 2.0 – 10.0 μ M, thereby allowing Ca^{2+} to bind to the myofilament protein troponin C resulting in sarcomere shortening and muscle contraction. Muscle relaxation is initiated by RyR2 closure accompanied by Ca^{2+} dissociation from the troponin complex and its reuptake into the SR by SERCA2a (75% of Ca^{2+} removal in human) its removal by the sarcolemmal Na^{2+}/Ca^{2+} exchanger (NCX) (25% of Ca^{2+} removal in human). SERCA pump serves a dual function: 1) to cause muscle relaxation by lowering cytosolic $[Ca^{2+}]$, and 2) to restore SR Ca^{2+} load necessary for muscle contraction. SERCA2a enzymatic activity is controlled by the inhibitory peptide phospholamban (PLN). In its dephosphorylated form, PLN inhibits SERCA2a activity by lowering its affinity for Ca^{2+} .

The system of Ca^{2+} cycling is finely regulated by the β -adrenergic signaling system through phosphorylation and dephosphorylation (Figure 2). In response to stress, binding of agonists to β -Adrenergic receptors (β ARs) results in adenylyl cyclase (AC) stimulation, cAMP production and activation of protein kinase A (PKA). PKA phosphorylates LTCC and RyR, thus increasing cytosolic Ca^{2+} influx, whereas phosphorylation of troponin-I increases the overall contractility, mediating the inotropic and chronotropic effect of β AR. Phosphorylation of PLN on serine-16 by PKA relieves its inhibition of SERCA2a and contributes to a frequency-dependent acceleration of SR calcium reuptake, mediating the lusitropic effect of β ARs. An additional critical target in calcium cycling is inhibitor-1 (I-1).

I-1 becomes active upon PKA phosphorylation and inhibits the type 1 serine/threonine protein phosphatase (PP1) resulting in amplification of β AR responses in the heart¹⁵ (Figure 2).

Chronic HF is associated with increased sympathetic outflow, which may be compensatory early in the disease state; however long-term neurohormonal activation induces significant damage to cardiomyocytes. Long-term chronic stimulation of β ARs results in multiple alterations in the β AR signaling cascade including β AR down regulation, upregulation of β AR kinase (β ARK) and increased inhibitory G-protein α -subunit function^{3, 13}. In failing cardiomyocytes the intracellular $[Ca^{2+}]$ is elevated, the amplitude of the $[Ca^{2+}]$ transient is decreased, and its duration is prolonged. These significant alterations in Ca^{2+} handling along with SR Ca^{2+} leak through the RyR, decreased SR Ca^{2+} uptake and decreased SR Ca^{2+} content, result in abnormal excitation-contraction coupling^{3, 5}.

The importance of SERCA2a in HF has been studied extensively in both animal models and in humans. These studies have shown that SERCA expression and activity is reduced in failing myocardium^{3, 5}. Increased inhibitory function of PLN¹⁶ and increased activity of PP1 resulting in dephosphorylation and inactivation of PKA targets¹⁵ also contribute to impaired SR Ca^{2+} uptake. The common finding is a substantial decrease in SR Ca^{2+} uptake which is a crucial component of the impaired contractile performance of the failing heart.

In many cases, altered Ca^{2+} cycling precedes the observed depression of mechanical performance; consequently, improving of Ca^{2+} cycling has potential as an attractive strategy against HF^{3, 5}.

2. Effect of SERCA2a gene transfer on calcium regulation and cell functions

Calcium is an ubiquitous and pleiotropic second messenger, thus, modulation of calcium signaling by SERCA2a gene transfer can affect multiple functions in different cardiovascular cell types. At least 11 isoforms of SERCA encoded by three separate homologous genes (ATP2A1, ATP2A2 and ATP2A3) have been discovered today^{7, 17, 18}. Given the functional differences between isoforms, only cardiac specific isoform SERCA2a is therapeutically useful, others are or may be harmful or ineffective^{19–21}.

Contractility

Adenovirus-mediated gene transfer of SERCA2a to failing human cardiomyocytes restored the calcium transient and improved contraction and relaxation velocity to the level of non-failing myocytes²². The enhanced contractility induced by SERCA2a overexpression was due to an enhanced SR Ca^{2+} uptake during diastole, increased SR Ca^{2+} content, and more effective Ca^{2+} efflux during systole (Figure 3).

Mechanoenergetics

Calcium overload leading to increased calcium uptake by mitochondria, resulted in mitochondrial dysfunction and decreased ATP production^{23, 24}. Normalization of intracellular calcium by SERCA2a overexpression should prevent mitochondrial dysfunction and decrease energy cost for Ca^{2+} regulation. Sakata *et al.* investigated the oxygen cost of left ventricular (LV) contractility, obtained by plotting myocardial oxygen consumption per beat against equivalent maximal elastance^{8, 9}. In diseased hearts, the oxygen cost of LV contractility was increased; SERCA2a gene transfer restored the increased oxygen cost to the normal level. In addition, restoring SERCA2a was shown to correct the creatine kinase activity by normalizing the creatine to ATP ratio in animal model

of HF²⁵. Therefore SERCA2a gene transfer restores cardiac energetics both by improving mechanoenergetic efficiency and by enhancing energy supply.

Arrhythmias

Diastolic Ca^{2+} overload occurring in ischemia and chronic HF is responsible for cellular electric instability resulting in tachyarrhythmias⁵. The increase in Ca^{2+} ions in the cell and SR leads to generation of asynchronous Ca^{2+} waves during diastole via abnormal activity of RyRs. These waves activate the electrogenic NCX and thereby produce arrhythmogenic spontaneous aftercontractions and afterdepolarizations²⁶. Recent data demonstrated that SR Ca^{2+} load via SERCA2a is responsible to sensitization of RyR, thereby creating a traveling wave of high Ca^{2+} sensitivity initiating Ca^{2+} release²⁷. SERCA2a overexpression in rabbit cardiomyocytes, favoring sequestration of Ca^{2+} in the SR, result in rapid inactivation of subsequent Ca^{2+} currents and reduced Ca^{2+} entry through LTCC²⁸. In this model, reduction of trans-sarcolemmal Ca^{2+} flux by SERCA2a overexpression without changes of normal systolic transient also reduced the occurrence of aftercontractions and afterdepolarization²⁸. As such, reduction of cytosolic Ca^{2+} overload might constitute a viable strategy for combating arrhythmia (Figure 4). In support of this hypothesis, Del Monte et al. showed that SERCA2a gene transfer in a model of ischemia/reperfusion injury effectively suppressed arrhythmias and reduced myocardial infarct size¹⁰.

Transcription

Calcium also acts on Ca^{2+} -dependent transcription pathways via the activation of various kinases and phosphatases⁶ (Figure 5). The Ca^{2+} -dependent serine/threonine phosphatase calcineurin was identified as a central pro-hypertrophic signaling molecule in the myocardium²⁹. Its target, nuclear factor of activated T lymphocytes (NFAT), was shown to be necessary and sufficient for mediating pathological hypertrophic remodeling³⁰ apoptosis³¹ and inflammation³² in cardiomyocytes and proliferation of VSMC¹². During hypertrophic /proliferative remodeling, reduced SR Ca^{2+} store and increased expression of transient receptor potential channels (TRPC)⁷ favor store-operated Ca^{2+} entry and sustained activation of calcineurin. In VSMC overexpression of SERCA2a inhibits calcineurin activity and NFAT-driven gene transcription¹².

Hypertrophy Proliferation

Myocardial hypertrophy is an adaptive response to hormonal and mechanical stimuli. Several reports suggest that SERCA2a overexpression rescues or prevents cardiac hypertrophy^{33, 34}. Although the mechanism of this benefit was not completely elucidated in the heart, it is speculated that they may result from inhibition of calcineurin, as shown in VSMC¹². In VSMC SERCA2b (ubiquitous isoform) and SERCA2a are co-expressed in adulthood. During proliferation VSMC undergo de-differentiation and acquisition of a synthetic phenotype, associated with loss of SERCA2a. Restoring SERCA2a expression in VSMC by gene transfer inhibits VSMC proliferation of and post-injury restenosis in a rat carotid injury model¹².

NO synthesis

NO synthesis is controlled by intracellular Ca^{2+} oscillations, and increase in SERCA activity by overexpression of S100A1 (see below) also increases NO production in endothelial cells (EC)³⁵. Sakata et al showed that transcortical gene transfer of SERCA2a increase coronary blood flow³⁴. One of the reasons for this improvement is the increased production of NO caused by SERCA2a overexpression in EC¹¹. This additional effect of SERCA2a overexpression might have a synergic benefit on cardiovascular disease.

3) SERCA2a as a target for heart failure gene therapy in animal models

Several studies have been designed to improve cardiac function using SERCA as a target. The SERCA isoform used is an important consideration. Data from transgenic animals demonstrated functional differences between the isoforms SERCA2a and SERCA2b in the heart. Long-term expression of SERCA2a in transgenic animals improves contractility without adverse effects³⁶, but replacement of SERCA2a by SERCA2b isoform resulted in cardiac dysfunction and hypertrophy^{19, 20}. Adenovirus-mediated gene transfer of SERCA2a improved cardiac hemodynamics and increased survival in a rat model of HF²⁵, whereas gene transfer of the faster skeletal muscle isoform SERCA1 had limited functional and metabolic improvement in the same model and compromised function in hypertrophic hearts²¹.

SERCA2a has been the subject of most gene therapy approaches targeting altered calcium physiology⁵. The increase in contractility concomitant with an increase in SERCA2a expression has been shown by gene transfer and transgenic methods in healthy, HF or diabetes mellitus animals and by gene transfer in a large animal model of HF^{5, 37}. Furthermore, SERCA2a gene transfer restored the oxygen cost of LV contractility to the normal level, indicating an improvement in energy utilization^{8, 9, 25}.

LV dysfunction is a major predictor of electrical instability and arrhythmias. Hence, improvement of myocardial contractility by gene transfer could have a major beneficial electrophysiological effect. Adenovirus-directed gene transfer of SERCA2a in a rat model of ischemia/reperfusion injury effectively suppressed arrhythmias and reduced myocardial infarct size¹⁰. In contrast with this finding, overexpression of SERCA2a in transgenic rats increased the occurrence of fatal ventricular tachyarrhythmia following infarct³⁸. These latter finding could result from adverse electrical remodeling caused by reduced NCX expression and function triggered by enhanced SR Ca^{2+} content. Overall, the overexpression of SERCA2a by gene transfer in the setting of ischemia reperfusion both in small and large animals has been shown to reduce ventricular arrhythmias^{10, 39}.

Recently several preclinical HF studies were conducted on large animal models. A porcine model of HF was created by mitral regurgitation⁴⁰. AAV1/SERCA2a was administrated using single intracoronary perfusion. Two months after treatment SERCA2a-injected animals demonstrated significant improvements in contractile function and restoration of ventricular volumes compared with untreated animals. There was an absolute increase of 16% in median ejection fraction in AAV1/SERCA2a treated animals⁴⁰. A sheep model of HF was created by rapid pacing³⁷. Delivery of AAV1/SERCA2a by cardiac recirculating elicited a dose-dependent improvement in LV function 1–2 month after virus administration³⁷. In both studies, safety-related parameters including histopathologic analysis, hematology, and clinical chemistry indicate that the treatment did not cause any organ damage or inflammatory response^{37, 40}. Furthermore, in addition to favorable haemodynamic effects, brain natriuretic peptide (BNP) expression was reduced consistent with reversal of the HF molecular phenotype^{37, 40}. To evaluate the potential toxicological effect of AAV1/SERCA2 gene therapy the virus was administrated using transcatheter perfusion in normal Göttingen minipigs⁴¹. The animals received the dose at least 3-fold higher than the highest dose intended for administration in clinical trials. AAV1/SERCA2a DNA was found in the majority of all the tissues sampled 5 days after injection. The highest concentrations were found in the lung and liver, low to non detectable levels of AAV1/SERCA2a were found in blood and brain tissue. Given the wide spread level of “junk” DNA in the human genome, the safety consequence of the presence of vector DNA in non cardiac cells such as liver is expected to be minimal or nonexistent⁴¹. On Days 30 to 90, the tissue concentrations of AAV1/SERCA2a decreased significantly, with many tissues having low to

undetectable concentrations of AAV1/SERCA2a. On 90 days after gene transfer, there was an increase in SERCA2a protein expression in the left and right ventricular wall; in addition, coronary arteries, pulmonary arteries, and the diaphragm and lung tissues showed increased expression of SERCA2a, without apparent toxicologic effect⁴¹. However, there was no change in SERCA2 expression in liver, kidney, spleen, retina, biceps, sartorius, and gastrocnemius muscles⁴¹. AAV1/SERCA2a was well tolerated in minipigs; no signs of toxicity, clinical pathology or histology were observed, and values for echocardiography and electrocardiography parameters were found to be within normal range⁴¹.

The overall conclusion from animal studies is that AAV-directed SERCA2a gene transfer appears to be a safe, well-tolerated, and effective treatment for HF.

4. Small molecules targeting SERCA activity

Phospholamban

Inhibition of PLN is another therapeutic approach to target the Ca^{2+} handling pathway in the failing heart^{3, 13}. Gene transfer of a dominant-negative PLN mutant⁴², PLN antisense RNAs⁴³ and intracellular inhibitory PLN antibodies⁴⁴ have been used to increase SERCA2a activity and to rescue HF in animal models. Furthermore, normalization of the calcium transient and restoration of cell contractility has been reported in cardiomyocytes isolated from failing human hearts after adenovirus-mediated PLN antisense gene transfer^{45, 46}. Recently, successful treatment of HF was demonstrated in a rat model of aortic banding by RNAi targeting of PLN⁴⁷. Simple intravenous injection of PLN RNAi carried by rAAV9 provided long-term stable cardiac shPLN expression and restores cardiac function, reduces dilatation, hypertrophy and fibrosis after 3 months⁴⁷. The excitement generated by these studies has been tempered by the discovery in human two mutations on PLN, leading to PLN-null genotype in homozygous individuals with a phenotype of dilated cardiomyopathy^{48–50}. These latter developments raise doubts about using PLN ablation as a universal approach to treat all forms of HF.

Inhibitor 1

Targeting PP1 by overexpression of its inhibitory peptide (I-1) to increase SERCA2a activity may constitute a promising therapeutic strategy in HF^{13, 15}. Indeed, silencing PP1 activity in transgenic mice by the expression of a constitutively active I-1 protein (I-1_{T35D}) results in enhanced PLN phosphorylation, augmented cardiac contractility, rescues from HF development upon aortic constriction and improves mechanical recovery and survival in an ischemia/reperfusion model^{51, 52}. Similarly, adenovirus gene transfer of I-1_{T35D} results in marked restoration of contractility in animal models and failing human myocytes⁵¹.

S100A1

Another important protein that has been recently targeted is S100A1, a low-molecular weight Ca^{2+} binding protein that stabilizes RyR and increases SERCA ATPase activity, resulting in a balanced enhancement of SR Ca^{2+} release and uptake^{13, 53}. In HF the expression of S100A1 is reduced. Gene transfer of S100A1 in rats following cryo-infarction induced an increase in SERCA2a and RyR activities, resulting in improved intracellular calcium handling and overall enhanced systolic and diastolic ventricular function^{54, 55}.

Pharmacotherapy

Recently, a novel inotropic agent that enhances SERCA2a activity was discovered and tested in HF models. Istaroxime, (E, Z)-3-((2-aminoethoxy)imino) androstane-6,17-dione hydrochloride) enhances SR Ca^{2+} content and Ca^{2+} uptake in failing cardiomyocytes and

improves heart function in a guinea pig aortic binding model^{56, 57}. This finding opens new perspective for the pharmacotherapy of HF.

5. Advances in the development of viral vectors

The molecular vectors for cardiac gene therapy generally employ expressing cassettes containing strong virus promoters that are constitutively active in a wide spectrum of cells (e.g. cytomegalovirus promoter [CMV]), the gene of interest (SERCA2a) and an appropriate mRNA stabilizing polyadenylation signal. The most commonly used virus vectors are recombinant adeno- and adeno-associated viruses (Table 1).

Adenoviruses

Recombinant human adenovirus (Ad) vectors are used essentially in preclinical small animal models. Adenoviruses are non-enveloped vectors that are very efficient at transducing target cells *in vitro* and *in vivo*, and can be prepared at high titers. The wild type Ad genome is approximately 35 kb of which up to 30 kb can be replaced with foreign DNA. These vectors have demonstrated efficient transduction of adult myocardium *in vivo* resulting in alteration of global heart function. In animal models, Ads mediate high-level expression for only short term (1 week). However, Ad cause intense local inflammation and stimulate potent cellular and humoral immune responses¹⁴.

Adeno-associated viruses

Recombinant adeno-associated viruses vectors (rAAV), derived from nonpathogenic parvoviruses, have many characteristics that make them particularly well suited for cardiac gene transfer⁵⁸. First, AAV effectively transfect dividing and non-dividing cells. Second, AAV provide stable long term (up to a year) expression in most systems⁵⁹. Third, AAV evoke minimal immune response in humans and are not known to cause human disease. AAV vectors have been studied in hundreds of patients, and have demonstrated an excellent safety profile^{14, 60}.

Major limitation of rAAV vector systems is the limited packaging capacity of 4.8 kb. AAV vectors package single- stranded genomes and require host cell synthesis of complementary strand for transduction. Recently, a new AAV vector (self-complementary [sc]AAV) which can package either two copies of DNA or dimeric-inverted repeat DNA molecules, thereby alleviating the requirement for host-cell DNA synthesis, was developed^{61, 62}. However, the cassette sizes have to be half the size of a normal sized single-stranded vector (i.e., 2.3 kb), which further limits the number of genes that can be used with scAAV.

Today, 12 different AAV serotypes are known, each with different tissue tropisms⁶³. AAV1, 2, 6, 8 and 9 have higher tropism for myocardium^{64–66}. AAV8 and 9 efficiently cross the endothelial barrier and can be injected intravenously or intraperitoneally^{65, 67}. New mutant AAV pseudotypes have been constructed recently, to improve tissue specificity and safety of gene transfer^{58, 68}. The pseudotype used in human Phase I clinical trial, AAV2/1, contains the capsid sequence of AAV1 and the inverted terminal repeats (ITR) sequences of AAV2. The AAV2 ITRs are incorporated because they have been used in many previous clinical trials and their safety profile is established⁶⁸. The AAV2/1 vector was selected because AAV1 has been shown to be superior to the other AAV serotypes in transducing cardiomyocytes⁶⁴. Newly constructed pseudotypes rAAV2/8 and rAAV2/9 are able to transduce myocardium of neonatal mice at approximately 20- and 200-fold (respectively) higher levels than rAAV2/1^{47, 65}.

Thus, recombinant rAAV vectors hold a great promise for delivering genes to treat heart diseases.

6. Techniques of Gene Delivery

A number of mechanical approaches have been developed for cardiac gene delivery including direct intramyocardial injection, endocardial delivery, ventricular cavity infusion during aortic cross-clamping, pericardial delivery and percutaneous coronary catheterization^{3, 5}.

Direct intramyocardial injection let to efficient local gene delivery, but was often limited by a patchy pattern of vector transfer and procedure related risks^{69, 70}. Intravenous delivery requires injection of at least 10^{11} AAV genomes to initiate transduction in small animals and is not applicable to human diseases. The best method developed for the rat model involves injecting the virus into the aortic root just above the aortic valve, while the aorta and pulmonary artery are transiently cross-clamped, achieving homogeneous transduction of the myocardium⁷¹.

Endocardial delivery is limited to focal gene transfer to the myocardium. This method of vector delivery for therapeutic angiogenesis has been using in Phase I clinical trials of patients with medically refractory severe angina⁷².

Intracoronary delivery or retrograde infusion of the virus via the coronary veins results are the attractive solutions since all of the myocardial territory supplied by a coronary artery is accessible. Percutaneous catheter-based gene delivery to the myocardium *in vivo* can be achieved by the endocardial or the intracoronary veins delivery by several approaches:

1. Antegrade Epicardial Coronary Artery Infusion (AECAL). The vector is slowly infused into the coronary arteries⁴².
2. Modified percutaneous antegrade myocardial gene transfer (PAMGT). Catheter-based antegrade intracoronary viral gene delivery with coronary venous blockade⁷³.
3. V-Focus Cardiac Delivery System. The V-Focus system is an investigational device that isolates the coronary circulation from the remainder of the systemic circulation while maintaining perfusion of the myocardium⁷⁴. The V-Focus system enables a closed circuit to be percutaneously established between the coronary arteries and coronary sinus in which the virus vector circulates through the myocardium for approximately 10 min.

The catheter-based techniques were developed for cardiac gene delivery in large animals and may be amenable to translation into human.

7. Clinical trials of heart failure using SERCA2a

The central role of SERCA2a in human HF has been established in patients treated with heart transplant, drugs and devices. Diastolic dysfunction in heart transplant recipients has been shown associated with decreased SERCA2a expression⁷⁵. Improved LV ejection fraction in DCM (dilated cardiomyopathy) patients treated with β -blockers was correlated with increases in SERCA2a mRNA concentrations in myocardial biopsies⁷⁶. Patients with DCM and end-stage HF in whom combined LV assist device and pharmacological therapy induced sufficient clinical recovery to allow removal of the device without recurrence of HF, had increased SR Ca^{2+} content compared with patients who did not recover⁷⁷. In patients (n=11) with advanced CHF, functional improvement related to CRT (cardiac resynchronization therapy) was associated with favorable changes in molecular markers of HF, including increase in SERCA2a mRNA; no significant changes were observed in non responders patients⁷⁸. Improved symptoms and exercise tolerance in HF patients after 3

months treatment with Cardiac Contractility Modulator (CCM) has been associated with increase in SERCA2a mRNA^{79, 80}.

Following a long line of investigation on animal models, gene transfer of SERCA2a has shown tremendous promise and two clinical trials using SERCA2a as a target have been initiated: a Phase I, randomized, double-blinded, placebo-controlled, dose escalation trial of intracoronary administration of AAV1. SERCA2a (MYDICAR™) in patients with CHF (Celladon Corp., CA, USA) and a Phase I study evaluating the safety and biological effects of AAV6. SERCA2a in non-ischemic patients undergoing left ventricular assist device placement.

The investigational agent (MYDICAR™) used in the first clinical trial⁴¹ is a rAAV1 vector and consists of a single-stranded 4486 nucleotide DNA containing the human SERCA2a expression cassette (CMV promoter- SERCA2a-bovine growth hormone pA signal) flanked by ITRs derived from AAV2. The MEDRAD Mark V ProVis angiographic injection system is used to produce a slow, diffuse, and homogenous myocardial exposure to AAV1/SERCA2a via AECAI over 10 min⁷⁴.

The objectives of phase I clinical trial are the following: 1) to evaluate the safety and feasibility of a 1 time AECAI administration of MYDICAR™ to subjects with ischemic or nonischemic DCM and Class III/IV symptoms of HF; 2) to explore the activity/efficacy of MYDICAR™ and identify appropriate doses for future studies⁴¹.

The method of cardio-specific vector administration chosen for human testing is AECAI⁴². Vector is slowly infused into the coronary arteries after percutaneous access obtained using a femoral approach. Laboratory assessment is monitored for 12 months after administration of AAV1/SERCA2a and includes following parameters: assessment of cardiac enzymes (creatinase kinase-MB and cardiac troponin; BNP and N-terminal prohormone brain natriuretic peptide (NT-proBNP); ELISPOT assay to assess possible anti-AAV1 capsid T-cell response; anti-AAV1 neutralizing antibody assay. Sequential echocardiograms are used to assess response to therapy. Global systolic and diastolic functions are analyzed. Parameters measured include left ventricular sizes and volumes. The primary safety endpoint is based on the Stage 1 12-months follow-up data, the primary activity/efficacy analysis is based on the Stage 2 6-month postdose data.

In the phase 1 trial a total of 12 patients with advanced HF have received intracoronary infusion of AAV1.SERCA2a⁸¹. Of the 9 reported patients treated, several demonstrated improvements from baseline to month 6 of a number of HF parameters⁸¹. Of note, 2 patients who failed to improve had preexisting anti-AAV-1 antibodies.

Conclusion

Advances in the understanding of the molecular basis of HF together with the evolution of increasingly efficient gene transfer technology, has finally placed congestive HF within reach of gene-based therapy. The intracellular handling of calcium is central to the process of excitation-contraction coupling and a number of calcium cycling proteins have been involved in the pathogenesis of HF. Of these, increasing SERCA2a function have been the subject of a large number of gene therapy approaches targeting calcium physiology. Beyond its beneficial effects on contractile function, SERCA2a had profound effect on hypertrophic remodeling, mechanoenergetics, arrhythmogenesis, vascular smooth muscle and endothelial function. These additional effects have heightened the interest in this versatile Ca²⁺ transporter. However the majority of SERCA2a studies have used animal models, which do not precisely mirror human HF. The patients with end-stage HF may have a greater degree of fibrosis or chronic oxidative stress, reducing efficacy of gene therapy. Early results in the

Phase I of a first in-human study of AAV1/SERCA2a in advanced HF showed improvement of a number of HF parameters. Further human studies are greatly needed. Phase 2 of this trial which is placebo controlled randomized and blinded with three therapeutic arms and one control arm is currently under way with more than 30 patients having been enrolled. The results of this important phase 2 trial will be available in 2010.

Expert opinion

The clinical cardiovascular gene therapy experience is very limited. The available data, however, emphasize the importance of randomized placebo-controlled trials in assessing the efficacy of novel treatments. Larger trials of this type are in progress and the results of these are eagerly awaited. Meanwhile, the large volume of preclinical data is likely to continue to expand as knowledge of the molecular basis of cardiovascular disease increases. Importantly, the accumulation of this data provides the scientific basis from which the human evaluation of novel gene-based therapies can be pursued.

With regard to gene transfer technology, recent serious adverse reactions in young human subjects have been attributed to the vector employed. As a result, efforts to improve vector biosafety have been revitalized; vectors will undergo modifications to further improve biosafety and gene transfer efficiency. Conventional treatment modalities have made significant advances over the last decade in the areas of pharmacotherapy, vascular intervention, surgery and implantable devices. The modest success of gene therapy in cardiovascular disease to date has been in filling a therapeutic need. Future success will depend on either improving conventional therapies or finding effective gene-based therapies. In the next few years, a number of Phase I clinical trials using AAV.SERCA2a based gene therapy for heart failure will provide much needed new information in this field.

Essential to the success of the clinical gene therapy trials in heart failure is the ability to safely use AAV vectors targeting the heart. Even though AAV vectors have been proven to be safe in our trials in patients with heart failure, in our recent experience with the clinical gene therapy trials, we have found that their use for gene delivery has the following limitations: 1) they are not specific for the heart and 2) pre-existing neutralizing antibodies to any individual serotype result in 40% of exclusion of these patients from clinical trials. To identify hybrid viruses that can selectively transduce cardiomyocytes *in vivo*, and which can also escape the antecedent neutralizing antibodies, a number of groups have been employing a combinatorial approach to generate a library of diverse AAV variants, obtained by DNA shuffling, with an enrichment of cardiotropic AAV variants, by directed evolution^{82, 83}

This has allowed the generation of cardiotropic chimerics of AAVs that more specifically targets the heart and escapes the inherent immunity in patients. In the next ten years, this approach will be used to generate novel cardiotropic AAV derived vectors that can also escape patients neutralizing antibodies.

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Abbreviations

α AR	α -adrenergic receptor
Ca ²⁺	calcium ion

CaM	calmodulin
CaMK	Ca ²⁺ /CaM – dependent protein kinase
DAG	Diacylglycerol
ET-1	Endothelin-1 receptor
Gαq	Gq protein α-subunit
HDAC	Histone deacetylase
IP3	Inositol-1,4,5-triphosphate
IP3R	IP3 receptor
Ica	L-type calcium current
LTCC	L-type calcium channel
MEF	Myocyte-enhancing factor
NFAT	Nuclear Factor of Activated T lymphocytes
Orai	SOC sub-unite
P	Phosphorylation
PLC	Phospholipase C
PLN	Phospholamban
PP2B	serine/threonin phosphatase 2B, calcineurin
RyR	Ryanodine Receptor calcium channel
SR	Sarcoplasmic Reticulum
SERCA	Sarco/Endoplasmic Reticulum Ca ²⁺ ATPase 2a
SRF	Serum Response Factor
TRPC	Transient Receptor Potential Ca ²⁺ channel, SOC sub-unite
STIM 1	stromal interacting molecule 1, SR calcium sensor., SOC sub-unite

Bibliography

Papers of special note have been highlighted as either of interest (*) or of considerable interest (**).

1. Rosamond W, Flegal K, Furie K, Go A, Greenlund K, Haase N, et al. Heart disease and stroke statistics--2008 update: a report from the American Heart Association Statistics Committee and Stroke Statistics Subcommittee. *Circulation* Jan 29;2008 117(4):e25–146. [PubMed: 18086926]
2. Jessup M, Brozena S. Heart failure. *N Engl J Med* May 15;2003 348(20):2007–18. [PubMed: 12748317]
3. Lipskaia L, Ly H, Kawase Y, Hajjar R, Lompre AM. Treatment of heart failure by calcium cycling gene therapy. *Future Cardiology* 2007;3(4):413–23. [PubMed: 19804232]
4. Del Monte F, Hajjar RJ. Intracellular devastation in heart failure. *Heart Fail Rev* Jun;2008 13(2): 151–62. [PubMed: 18347978]
5. Kawase Y, Hajjar RJ. The cardiac sarcoplasmic/endoplasmic reticulum calcium ATPase: a potent target for cardiovascular diseases. *Nat Clin Pract Cardiovasc Med* Sep;2008 5(9):554–65. [PubMed: 18665137] **Review on calcium cycling gene therapy
6. Bers DM. Calcium cycling and signaling in cardiac myocytes. *Annu Rev Physiol* 2008;70:23–49. [PubMed: 17988210] **Well -documented review on calcium cycling.

7. Lipskaia L, Hulot JS, Lompre AM. Role of sarco/endoplasmic reticulum calcium content and calcium ATPase activity in the control of cell growth and proliferation. *Pflugers Arch* Jan;2009 457(3):673–85. [PubMed: 18188588] *Calcium-regulated transcription pathways
8. Sakata S, Lebeche D, Sakata N, Sakata Y, Chemaly ER, Liang LF, et al. Restoration of mechanical and energetic function in failing aortic-banded rat hearts by gene transfer of calcium cycling proteins. *J Mol Cell Cardiol* Apr;2007 42(4):852–61. [PubMed: 17300800]
9. Sakata S, Lebeche D, Sakata Y, Sakata N, Chemaly ER, Liang LF, et al. Mechanical and metabolic rescue in a type II diabetes model of cardiomyopathy by targeted gene transfer. *Mol Ther* May;2006 13(5):987–96. [PubMed: 16503203]
10. del Monte F, Lebeche D, Guerrero JL, Tsuji T, Doye AA, Gwathmey JK, et al. Abrogation of ventricular arrhythmias in a model of ischemia and reperfusion by targeting myocardial calcium cycling. *Proc Natl Acad Sci U S A* Apr 13;2004 101(15):5622–7. [PubMed: 15044708]
11. Hadri L, Lipskaia L, Kawase Y, Clement N, Plenge T, Lebeche D, et al. Transcoronary gene transfer of SERCA2a increases coronary blood flow through an increase of eNOS activity in endothelial cells. *Circ Res* 2007;101:E65–E.
12. Lipskaia L, del Monte F, Capiod T, Yacoubi S, Hadri L, Hours M, et al. Sarco/endoplasmic reticulum Ca²⁺-ATPase gene transfer reduces vascular smooth muscle cell proliferation and neointima formation in the rat. *Circ Res* Sep 2;2005 97(5):488–95. [PubMed: 16081870]
13. Vinge LE, Raake PW, Koch WJ. Gene therapy in heart failure. *Circ Res* Jun 20;2008 102(12):1458–70. [PubMed: 18566312] **Well documented review on HF gene therapy
14. Lyon AR, Sato M, Hajjar RJ, Samulski RJ, Harding SE. Gene therapy: targeting the myocardium. *Heart Jan;2008* 94(1):89–99. [PubMed: 18083952] **Well documented review on HF gene therapy
15. Nicolaou P, Hajjar RJ, Kranias EG. Role of protein phosphatase-1 inhibitor-1 in cardiac physiology and pathophysiology. *J Mol Cell Cardiol*. May 27;2009 *Exhaustive review of inhibitor-1.
16. MacLennan DH, Kranias EG. Phospholamban: a crucial regulator of cardiac contractility. *Nat Rev Mol Cell Biol* Jul;2003 4(7):566–77. [PubMed: 12838339]
17. Bobe R, Bredoux R, Corvazier E, Lacabartz-Porret C, Martin V, Kovacs T, et al. How many Ca(2)+ATPase isoforms are expressed in a cell type? A growing family of membrane proteins illustrated by studies in platelets. *Platelets* May–Jun;2005 16(3–4):133–50. [PubMed: 16011958]
18. Periasamy M, Kalyanasundaram A. SERCA pump isoforms: their role in calcium transport and disease. *Muscle Nerve* Apr;2007 35(4):430–42. [PubMed: 17286271]
19. Vangheluwe P, Tjwa M, Van Den Bergh A, Louch WE, Beullens M, Dode L, et al. A SERCA2 pump with an increased Ca²⁺ affinity can lead to severe cardiac hypertrophy, stress intolerance and reduced life span. *J Mol Cell Cardiol* Aug;2006 41(2):308–17. [PubMed: 16814319]
20. Ver Heyen M, Heymans S, Antoons G, Reed T, Periasamy M, Awede B, et al. Replacement of the muscle-specific sarcoplasmic reticulum Ca(2+)-ATPase isoform SERCA2a by the nonmuscle SERCA2b homologue causes mild concentric hypertrophy and impairs contraction-relaxation of the heart. *Circ Res* Oct 26;2001 89(9):838–46. [PubMed: 11679415]
21. O'Donnell JM, Fields A, Xu X, Chowdhury SA, Geenen DL, Bi J. Limited functional and metabolic improvements in hypertrophic and healthy rat heart overexpressing the skeletal muscle isoform of SERCA1 by adenoviral gene transfer in vivo. *Am J Physiol Heart Circ Physiol* Dec; 2008 295(6):H2483–94. [PubMed: 18952713]
22. del Monte F, Harding SE, Schmidt U, Matsui T, Kang ZB, Dec GW, et al. Restoration of contractile function in isolated cardiomyocytes from failing human hearts by gene transfer of SERCA2a. *Circulation* Dec 7;1999 100(23):2308–11. [PubMed: 10587333]
23. Lipskaia L, Pinet C, Fromes Y, Hatem S, Cantaloube I, Coulombe A, et al. Mutation of delta-sarcoglycan is associated with Ca(2+) -dependent vascular remodeling in the Syrian hamster. *Am J Pathol* Jul;2007 171(1):162–71. [PubMed: 17591963]
24. Vandebrouck A, Ducret T, Basset O, Sebillé S, Raymond G, Ruegg U, et al. Regulation of store-operated calcium entries and mitochondrial uptake by minidystrophin expression in cultured myotubes. *FASEB J* Jan;2006 20(1):136–8. [PubMed: 16254044]

25. del Monte F, Williams E, Lebeche D, Schmidt U, Rosenzweig A, Gwathmey JK, et al. Improvement in survival and cardiac metabolism after gene transfer of sarcoplasmic reticulum Ca(2+)-ATPase in a rat model of heart failure. *Circulation* Sep 18;2001 104(12):1424–9. [PubMed: 11560860]
26. Venetucci LA, Trafford AW, O'Neill SC, Eisner DA. The sarcoplasmic reticulum and arrhythmogenic calcium release. *Cardiovasc Res* Jan 15;2008 77(2):285–92. [PubMed: 18006483]
27. Keller M, Kao JP, Egger M, Niggli E. Calcium waves driven by “sensitization” wave-fronts. *Cardiovasc Res* Apr 1;2007 74(1):39–45. [PubMed: 17336953]
28. Davia K, Bernobich E, Ranu HK, del Monte F, Terracciano CM, MacLeod KT, et al. SERCA2A overexpression decreases the incidence of aftercontractions in adult rabbit ventricular myocytes. *J Mol Cell Cardiol* May;2001 33(5):1005–15. [PubMed: 11343422]
29. Heineke J, Molkentin JD. Regulation of cardiac hypertrophy by intracellular signalling pathways. *Nat Rev Mol Cell Biol* Aug;2006 7(8):589–600. [PubMed: 16936699] *Transcription pathways in cardiac hypertrophy.
30. Wilkins BJ, De Windt LJ, Bueno OF, Braz JC, Glascock BJ, Kimball TF, et al. Targeted disruption of NFATc3, but not NFATc4, reveals an intrinsic defect in calcineurin-mediated cardiac hypertrophic growth. *Mol Cell Biol* Nov;2002 22(21):7603–13. [PubMed: 12370307]
31. Liu Q, Wilkins BJ, Lee YJ, Ichijo H, Molkentin JD. Direct interaction and reciprocal regulation between ASK1 and calcineurin-NFAT control cardiomyocyte death and growth. *Mol Cell Biol* May;2006 26(10):3785–97. [PubMed: 16648474]
32. Suzuki J, Bayna E, Li HL, Molle ED, Lew WY. Lipopolysaccharide activates calcineurin in ventricular myocytes. *J Am Coll Cardiol* Jan 30;2007 49(4):491–9. [PubMed: 17258096]
33. Nakayama H, Otsu K, Yamaguchi O, Nishida K, Date MO, Hongo K, et al. Cardiac-specific overexpression of a high Ca²⁺ affinity mutant of SERCA2a attenuates in vivo pressure overload cardiac hypertrophy. *FASEB J* Jan;2003 17(1):61–3. [PubMed: 12424227]
34. Sakata S, Lebeche D, Sakata Y, Sakata N, Chemaly ER, Liang L, et al. Transcoronary gene transfer of SERCA2a increases coronary blood flow and decreases cardiomyocyte size in a type 2 diabetic rat model. *Am J Physiol Heart Circ Physiol* Feb;2007 292(2):H1204–7. [PubMed: 17012346]
35. Pleger ST, Harris DM, Shan C, Vinge LE, Chuprun JK, Berzins B, et al. Endothelial S100A1 modulates vascular function via nitric oxide. *Circ Res* Apr 11;2008 102(7):786–94. [PubMed: 18292599]
36. He H, Giordano FJ, Hilal-Dandan R, Choi DJ, Rockman HA, McDonough PM, et al. Overexpression of the rat sarcoplasmic reticulum Ca²⁺ ATPase gene in the heart of transgenic mice accelerates calcium transients and cardiac relaxation. *J Clin Invest* Jul 15;1997 100(2):380–9. [PubMed: 9218515]
37. Byrne MJ, Power JM, Prevolos A, Mariani JA, Hajjar RJ, Kaye DM. Recirculating cardiac delivery of AAV2/1SERCA2a improves myocardial function in an experimental model of heart failure in large animals. *Gene Ther* Dec;2008 15(23):1550–7. [PubMed: 18650850]
38. Chen Y, Escoubet B, Prunier F, Amour J, Simonides WS, Vivien B, et al. Constitutive cardiac overexpression of sarcoplasmic/endoplasmic reticulum Ca²⁺-ATPase delays myocardial failure after myocardial infarction in rats at a cost of increased acute arrhythmias. *Circulation* Apr 20;2004 109(15):1898–903. [PubMed: 15037529]
39. Prunier F, Kawase Y, Gianni D, Scapin C, Danik SB, Ellinor PT, et al. Prevention of ventricular arrhythmias with sarcoplasmic reticulum Ca²⁺ ATPase pump overexpression in a porcine model of ischemia reperfusion. *Circulation* Aug 5;2008 118(6):614–24. [PubMed: 18645052]
40. Kawase Y, Ly HQ, Prunier F, Lebeche D, Shi Y, Jin H, et al. Reversal of cardiac dysfunction after long-term expression of SERCA2a by gene transfer in a pre-clinical model of heart failure. *J Am Coll Cardiol* Mar 18;2008 51(11):1112–9. [PubMed: 18342232]
41. Hajjar RJ, Zsebo K, Deckelbaum L, Thompson C, Rudy J, Yaroshinsky A, et al. Design of a phase 1/2 trial of intracoronary administration of AAV1/SERCA2a in patients with heart failure. *J Card Fail* Jun;2008 14(5):355–67. [PubMed: 18514926]

42. Kaye DM, Prevolos A, Marshall T, Byrne M, Hoshijima M, Hajjar R, et al. Percutaneous cardiac recirculation-mediated gene transfer of an inhibitory phospholamban peptide reverses advanced heart failure in large animals. *J Am Coll Cardiol* Jul 17;2007 50(3):253–60. [PubMed: 17631218]
43. Tsuji T, Del Monte F, Yoshikawa Y, Abe T, Shimizu J, Nakajima-Takenaka C, et al. Rescue of Ca²⁺ overload-induced left ventricular dysfunction by targeted ablation of phospholamban. *Am J Physiol Heart Circ Physiol* Feb;2009 296(2):H310–7. [PubMed: 19074672]
44. Dieterle T, Meyer M, Gu Y, Belke DD, Swanson E, Iwatate M, et al. Gene transfer of a phospholamban-targeted antibody improves calcium handling and cardiac function in heart failure. *Cardiovasc Res* Sep 1;2005 67(4):678–88. [PubMed: 15927173]
45. del Monte F, Harding SE, Dec GW, Gwathmey JK, Hajjar RJ. Targeting phospholamban by gene transfer in human heart failure. *Circulation* Feb 26;2002 105(8):904–7. [PubMed: 11864915]
46. Ziolo MT, Martin JL, Bossuyt J, Bers DM, Pogwizd SM. Adenoviral gene transfer of mutant phospholamban rescues contractile dysfunction in failing rabbit myocytes with relatively preserved SERCA function. *Circ Res* Apr 29;2005 96(8):815–7. [PubMed: 15790952]
47. Suckau L, Fechner H, Chemaly E, Krohn S, Hadri L, Kocksamper J, et al. Long-term cardiac-targeted RNA interference for the treatment of heart failure restores cardiac function and reduces pathological hypertrophy. *Circulation* Mar 10;2009 119(9):1241–52. [PubMed: 19237664]
48. Schmitt JP, Kamisago M, Asahi M, Li GH, Ahmad F, Mende U, et al. Dilated cardiomyopathy and heart failure caused by a mutation in phospholamban. *Science* Feb 28;2003 299(5611):1410–3. [PubMed: 12610310]
49. Haghighi K, Kolokathis F, Gramolini AO, Waggoner JR, Pater L, Lynch RA, et al. A mutation in the human phospholamban gene, deleting arginine 14, results in lethal, hereditary cardiomyopathy. *Proc Natl Acad Sci U S A* Jan 31;2006 103(5):1388–93. [PubMed: 16432188]
50. Haghighi K, Kolokathis F, Pater L, Lynch RA, Asahi M, Gramolini AO, et al. Human phospholamban null results in lethal dilated cardiomyopathy revealing a critical difference between mouse and human. *J Clin Invest* Mar;2003 111(6):869–76. [PubMed: 12639993]
51. Pathak A, del Monte F, Zhao W, Schultz JE, Lorenz JN, Bodi I, et al. Enhancement of cardiac function and suppression of heart failure progression by inhibition of protein phosphatase 1. *Circ Res* Apr 15;2005 96(7):756–66. [PubMed: 15746443]
52. Nicolaou P, Rodriguez P, Ren X, Zhou X, Qian J, Sadayappan S, et al. Inducible expression of active protein phosphatase-1 inhibitor-1 enhances basal cardiac function and protects against ischemia/reperfusion injury. *Circ Res* Apr 24;2009 104(8):1012–20. [PubMed: 19299645]
53. Most P, Remppis A, Pleger ST, Katus HA, Koch WJ. S100A1: a novel inotropic regulator of cardiac performance. Transition from molecular physiology to pathophysiological relevance. *Am J Physiol Regul Integr Comp Physiol* Aug;2007 293(2):R568–77. [PubMed: 17459908]
54. Most P, Pleger ST, Volkers M, Heidt B, Boerries M, Weichenhan D, et al. Cardiac adenoviral S100A1 gene delivery rescues failing myocardium. *J Clin Invest* Dec;2004 114(11):1550–63. [PubMed: 15578088]
55. Pleger ST, Most P, Boucher M, Soltys S, Chuprun JK, Pleger W, et al. Stable myocardial-specific AAV6-S100A1 gene therapy results in chronic functional heart failure rescue. *Circulation* May 15;2007 115(19):2506–15. [PubMed: 17470693]
56. Rocchetti M, Alemanni M, Mostacciuolo G, Barassi P, Altomare C, Chisci R, et al. Modulation of sarcoplasmic reticulum function by PST2744 [istaroxime; (E,Z)-3-((2-aminoethoxy)imino) androstane-6,17-dione hydrochloride] in a pressure-overload heart failure model. *J Pharmacol Exp Ther* Sep;2008 326(3):957–65. [PubMed: 18539651]
57. Micheletti R, Palazzo F, Barassi P, Giacalone G, Ferrandi M, Schiavone A, et al. Istaroxime, a stimulator of sarcoplasmic reticulum calcium adenosine triphosphatase isoform 2a activity, as a novel therapeutic approach to heart failure. *Am J Cardiol* Jan 22;2007 99(2A):24A–32A.
58. Gray SJ, Samulski RJ. Optimizing gene delivery vectors for the treatment of heart disease. *Expert Opin Biol Ther* Jul;2008 8(7):911–22. [PubMed: 18549322]
59. Samulski RJ. AAV vectors, the future workhorse of human gene therapy. *Ernst Schering Res Found Workshop* 2003;(43):25–40. [PubMed: 12894449]

60. Manno CS, Pierce GF, Arruda VR, Glader B, Ragni M, Rasko JJ, et al. Successful transduction of liver in hemophilia by AAV-Factor IX and limitations imposed by the host immune response. *Nat Med* Mar;2006 12(3):342–7. [PubMed: 16474400]
61. Choi VW, Samulski RJ, McCarty DM. Effects of adeno-associated virus DNA hairpin structure on recombination. *J Virol* Jun;2005 79(11):6801–7. [PubMed: 15890919]
62. McCarty DM, Fu H, Monahan PE, Toulson CE, Naik P, Samulski RJ. Adeno-associated virus terminal repeat (TR) mutant generates self-complementary vectors to overcome the rate-limiting step to transduction in vivo. *Gene Ther* Dec;2003 10(26):2112–8. [PubMed: 14625565]
63. Li W, Asokan A, Wu Z, Van Dyke T, DiPrimio N, Johnson JS, et al. Engineering and selection of shuffled AAV genomes: a new strategy for producing targeted biological nanoparticles. *Mol Ther* Jul;2008 16(7):1252–60. [PubMed: 18500254]
64. Palomeque J, Chemaly ER, Colosi P, Wellman JA, Zhou S, Del Monte F, et al. Efficiency of eight different AAV serotypes in transducing rat myocardium in vivo. *Gene Ther* Jul;2007 14(13):989–97. [PubMed: 17251988]
65. Pacak CA, Mah CS, Thattaliyath BD, Conlon TJ, Lewis MA, Cloutier DE, et al. Recombinant adeno-associated virus serotype 9 leads to preferential cardiac transduction in vivo. *Circ Res* Aug 18;2006 99(4):e3–9. [PubMed: 16873720]
66. Raake PW, Hinkel R, Muller S, Delker S, Kreuzpointner R, Kupatt C, et al. Cardio-specific long-term gene expression in a porcine model after selective pressure-regulated retroinfusion of adeno-associated viral (AAV) vectors. *Gene Ther* Jan;2008 15(1):12–7. [PubMed: 17943147]
67. Inagaki K, Fuess S, Storm TA, Gibson GA, McTiernan CF, Kay MA, et al. Robust systemic transduction with AAV9 vectors in mice: efficient global cardiac gene transfer superior to that of AAV8. *Mol Ther* Jul;2006 14(1):45–53. [PubMed: 16713360]
68. Schultz BR, Chamberlain JS. Recombinant adeno-associated virus transduction and integration. *Mol Ther* Jul;2008 16(7):1189–99. [PubMed: 18500252]
69. Wright MJ, Wightman LM, Lilley C, de Alwis M, Hart SL, Miller A, et al. In vivo myocardial gene transfer: optimization, evaluation and direct comparison of gene transfer vectors. *Basic Res Cardiol* May–Jun;2001 96(3):227–36. [PubMed: 11403416]
70. Muller OJ, Katus HA, Bekerredjian R. Targeting the heart with gene therapy-optimized gene delivery methods. *Cardiovasc Res* Feb 1;2007 73(3):453–62. [PubMed: 17097076]
71. Hajjar RJ, Schmidt U, Matsui T, Guerrero JL, Lee KH, Gwathmey JK, et al. Modulation of ventricular function through gene transfer in vivo. *Proc Natl Acad Sci U S A* Apr 28;1998 95(9):5251–6. [PubMed: 9560262]
72. Yla-Herttuala S. An update on angiogenic gene therapy: vascular endothelial growth factor and other directions. *Curr Opin Mol Ther* Aug;2006 8(4):295–300. [PubMed: 16955692]
73. Hayase M, Del Monte F, Kawase Y, Macneill BD, McGregor J, Yoneyama R, et al. Catheter-based antegrade intracoronary viral gene delivery with coronary venous blockade. *Am J Physiol Heart Circ Physiol* Jun;2005 288(6):H2995–3000. [PubMed: 15897329]
74. Prevolos A, Mennen M, Bilney A, Mariani J, Kaye D, Power J. Developpement of a novel perfusion technique to allow targeted delivery of Gene Therapyapy– the V-Focus system. *Journal of Extra-corporeal Technology* 2006;38:51–2. [PubMed: 16637525] *Gene delivery techniques for the heart
75. Studeli R, Jung S, Mohacsi P, Perruchoud S, Castiglioni P, Wenaweser P, et al. Diastolic dysfunction in human cardiac allografts is related with reduced SERCA2a gene expression. *Am J Transplant* Apr;2006 6(4):775–82. [PubMed: 16539635]
76. Lowes BD, Gilbert EM, Abraham WT, Minobe WA, Larrabee P, Ferguson D, et al. Myocardial gene expression in dilated cardiomyopathy treated with beta-blocking agents. *N Engl J Med* May 2;2002 346(18):1357–65. [PubMed: 11986409]
77. Terracciano CM, Hardy J, Birks EJ, Khaghani A, Banner NR, Yacoub MH. Clinical recovery from end-stage heart failure using left-ventricular assist device and pharmacological therapy correlates with increased sarcoplasmic reticulum calcium content but not with regression of cellular hypertrophy. *Circulation* May 18;2004 109(19):2263–5. [PubMed: 15136495]

78. Vanderheyden M, Mullens W, Delrue L, Goethals M, de Bruyne B, Wijns W, et al. Myocardial gene expression in heart failure patients treated with cardiac resynchronization therapy responders versus nonresponders. *J Am Coll Cardiol* Jan 15;2008 51(2):129–36. [PubMed: 18191736]
79. Butter C, Rastogi S, Minden HH, Meyhofer J, Burkhoff D, Sabbah HN. Cardiac contractility modulation electrical signals improve myocardial gene expression in patients with heart failure. *J Am Coll Cardiol* May 6;2008 51(18):1784–9. [PubMed: 18452785]
80. Mullens W, Bartunek J, Wilson Tang WH, Delrue L, Herbots L, Willems R, et al. Early and late effects of cardiac resynchronization therapy on force-frequency relation and contractility regulating gene expression in heart failure patients. *Heart Rhythm* Jan;2008 5(1):52–9. [PubMed: 18082469]
81. Jaski BE, Jessup ML, Mancini DM, Cappola TP, Pauly DF, Greenberg B, et al. Calcium upregulation by percutaneous administration of gene therapy in cardiac disease (CUPID Trial), a first-in-human phase 1/2 clinical trial. *J Card Fail* Apr;2009 15(3):171–81. [PubMed: 19327618]
82. Yang L, Jiang J, Drouin LM, Agbandje-McKenna M, Chen C, Qiao C, et al. A myocardium tropic adeno-associated virus (AAV) evolved by DNA shuffling and in vivo selection. *Proc Natl Acad Sci U S A* Mar 10;2009 106(10):3946–51. [PubMed: 19234115]
83. Koerber JT, Jang JH, Schaffer DV. DNA shuffling of adeno-associated virus yields functionally diverse viral progeny. *Mol Ther* Oct;2008 16(10):1703–9. [PubMed: 18728640]

Figure 1. Excitation-contraction coupling in cardiomyocytes

A small amount of Ca^{2+} uptake via LTCC causes a large amount of Ca^{2+} release from the SR via RyR – Ca^{2+} -induced Ca^{2+} release. Rise of intracellular Ca^{2+} causes contraction of myofilaments. Ca^{2+} uptake into the SR by SERCA or extrusion of Ca^{2+} through NCX channels results in relaxation of myofilaments. Abbreviations: Na-K ATPase: plasma membrane sodium-potassium ATPase; Ca^{2+} : calcium ion; Ica: L-type calcium current; LTCC: L-type calcium channel; K^{+} : potassium ion; PLN: Phospholamban; PMCA: Plasma Membrane Calcium ATPase; RyR: Ryanodine Receptor calcium channel; Na^{+} : sodium ion; NCX: - sodium-calcium exchanger; SR/ER: Sarco/Endoplasmic Reticulum; SERCA: Sarco/Endoplasmic Reticulum Ca^{2+} ATPase 2a.

Figure 2. The regulation of calcium cycling via adrenergic pathway and its alterations in failing heart

Schematic showing the positive control of cardiac function through classic β -adrenergic pathway and a negative control of cardiac function through the α -adrenergic pathway. In response to stress, binding of agonist to the β ARs results in inotrope (increased contractility), chronotrope (increased heart rate) and lusiotrope (enhanced relaxation) effects. These effects are mediated by PKA phosphorylation of several targets, resulting in enhanced Ca^{2+} cycling. Negative control of heart function through α ARs is mediated by PP1 dephosphorylation of several PKA targets. Abbreviations: α AR: α -adrenergic receptor; AC: Adenylyl cyclase; β AR: β -adrenergic receptor; β ARK: β -adrenergic receptor kinase; CIRC: Calcium induced calcium release; Gαq: Gq protein α -subunit; Gas: Stimulatory G-protein α -subunit; I-1: Inhibitor 1; Ica: L-type calcium current; LTCC: L-type calcium channel; P: Phosphorylation; PKA: Protein kinase A; PKC: Protein kinase C; PLN: Phospholamban; PLC: Phospholipase C; PP1: Serine/threonine phosphatase 1; RyR: Ryanodine receptor calcium channel; SERCA: Sarco/endoplasmic reticulum Ca^{2+} ATPase.

Figure 3. Effect of SERCA2a overexpression on contractile function in diseased heart

The overexpression of SERCA2a in failing hearts has been shown to result in the recovery of contractility. SERCA2a overexpression improves calcium cycling and myocyte shortening.

Abbreviations: CIRC: Calcium induced calcium release; L-type calcium current; LTCC: L-type calcium channel; PLN: Phospholamban; RyR: Ryanodine receptor calcium channel; SERCA: Sarco/endoplasmic reticulum Ca^{2+} ATPase.

Figure 4. Model showing the effect of SERCA2a overexpression on arrhythmias

The development of an unstable equilibrium is the mechanistic basis of increased arrhythmogenic risk. At the basal level (left) Ca²⁺ homeostasis is maintained by the balanced activities of LTCC and NCX, and between RyR2 and SERCA2a. Normal cardiac activation (middle) proportionally increases LTCC, NCX, RyR2 and SERCA function to maintain synchronized Ca²⁺ fluxes resulting in a stable state. Acute disruption of a single component relative to others (e.g. RyR) results in the ablation of synchronized Ca²⁺ fluxes and directly increases the arrhythmogenic propensity of the system (right). This state, illustrated using the experimentally observed findings of increased RyR2 leak and NCX activity and diminished SERCA activity, is inherently more prone to destabilization. SERCA2a gene transfer removes Ca²⁺ overload, stabilizes RyR2 and prevents arrhythmias. Abbreviations: Ca²⁺: calcium ion; LTCC: L-type calcium channel; PLN: Phospholamban; RyR: Ryanodine Receptor calcium channel; NCX: - sodium-calcium exchanger; SR: Sarcoplasmic Reticulum; SERCA: Sarco/Endoplasmic Reticulum Ca²⁺ ATPase 2a.

Figure 5. Model showing Ca^{2+} dependent hypertrophy/proliferation transcription pathways
Excitation/contraction calcium cycling is not involved in the control of hyperthrophic/proliferation pathways. Cardiac myocytes also contain lipid rafts rich in caveolin in the sarcolemma and T-tubules that are thought to generate local microdomains of Ca^{2+} . Activation of phosphoinositol-3-phosphate-coupled membrane receptors results in calcium release from the SR via IP_3R . Depletion of SR calcium close to the sarcolemma induces the formation of Store Operated Calcium complex resulted in calcium influx in local submembrane area. This calcium activates calcineurin (PP2B), which dephosphorylates NFAT , inducing its nuclear translocation and transcriptional activation.

Table 1

Comparison of major viral vector systems for cardiovascular gene transfer.

Vectors	Adenovirus	Adeno-Associated Virus
Functional titer (per ml)	Up to 10^{12}	Up to 10^{10}
Genome	dsDNA	ssDNA (can also be dsDNA)
Insert Capacity	7–30 kb	4.8 kb
Integration	No	Yes:chromosome 19 for wild-type AAV2 Low for recombinant vectors
Pattern of gene expression	Transient	Long-term
Cell cycle-dependent transduction	No	No
Immunoreactions	Cytotoxic and immunogenic	Minimally immunogenic
Clinical trial approved	Yes	Yes