REVIEW

Gene therapy for heart failure

Thérapie génique pour l’insuffisance cardiaque

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SUMMARY
Despite the progress achieved in conventional treatment modalities, heart failure remains a major cause of mortality and morbidity. The identification of novel signaling pathways has provided a solid scientific rationale which has stimulated preclinical development of gene-based therapies for heart failure. Advances in somatic gene transfer technologies have been crucial to the advent of the first human clinical trials which are currently in progress. As these and other trials of gene transfer-based therapies are initiated, these approaches have generated excitement and hope for novel treatments for cardiovascular disease. In this review, we present a summary of advancements in construction of different vectors and methods of delivery that have been used for specific myocardial gene delivery. In addition, we will show results from studies focusing on the use of gene therapy to target heart failure mechanisms in animal models of cardiac dysfunction. Finally, we discuss the limited but highly promising results from clinical studies that have served as catalysts to translate preclinical achievements towards new treatment modalities for heart failure.

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MOTS CLÉS
Thérapie génique; Insuffisance cardiaque

Résumé L’insuffisance cardiaque demeure une cause majeure de mortalité et de morbidité malgré le progrès dans les modalités de traitement conventionnel. L’identification de nouveaux mécanismes de signalisation a formé une base scientifique solide pour développer le traitement génétique de l’insuffisance cardiaque. Le succès des nouvelles technologies de transfert génétique a été crucial pour l’initiation des premiers essais cliniques qui sont actuellement en cours. Ces derniers génèrent beaucoup d’enthousiasme et un espoir de découvrir de nouvelles

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A genetic material surrounded by a capsid that interacts liposome-DNA and polymer-DNA complexes. They consist of clearly superior to non-viral vectors such as plasmid DNA, transgene expression. Viral vectors have been shown to be ure requires efficient myocardial transduction and long-term gene delivery. We will then discuss the different contemporary targets within the cardiac myocyte. The last part will present early results of the first in-human clinical trial (phase 1) of gene therapy in patients with HF. Far from being complete, this article emphasizes the most explored molecular techniques used in a field which, although remains in its infancy, promises to offer a breakthrough that might change the dismal history of heart failure.

### Viral vectors for gene transfer

Classical models of gene therapy rely on recombinant protein expression. In contrast, RNA interference (RNAi) therapy is a novel technology that uses short regulatory RNA sequences to modulate gene expression as its basic principle. Successful use of those strategies for heart failure requires efficient myocardial transduction and long-term transgene expression. Viral vectors have been shown to be clearly superior to non-viral vectors such as plasmid DNA, liposome-DNA and polymer-DNA complexes. They consist of a genetic material surrounded by a capsid that interacts with specific cell surface receptors to allow binding, internalization and delivery of the genome to the nucleus of the target cell. In this part, we will briefly review the characteristics of the three most commonly used viral vector families which are adenovirus, lentivirus and adeno-associated virus. Table 1 summarizes the advantages and limitations of each one of these families.

### Adenoviral vectors

Adenoviruses are double-stranded DNA viruses capable of carrying large genes of up to 30 kilobase pairs. They bind to the Coxsackie-Adenovirus receptors located on the plasma membrane and interact with cell surface integrins allowing viral endocytosis. Adenoviruses deliver and express their genomes within the nucleus of both dividing and non-dividing cells. Recombinant human adenoviruses are relatively cheap to produce in high titers and have a broad tropism to target cells especially within the cardiovascular system, which makes them widely used in myocardial gene therapy.

However, the use of adenoviruses is limited by a significant immune response that results in clearance of the transduced cells [1] and transient gene expression lasting 10 to 14 days in animal models [2]. The immune reaction destructs the cells enclosing the virus which in practice can result in adenoviral-induced myocarditis [3]. Adenoviruses have also an innate tropism to multiple human tissues which can result in transfection of unintended host tissues. Multiple side effects including fever, chills, shivering, myalgias and even death are reported in clinical trials [4]. Another major disadvantage is the presence of either preexisting or de novo formed antibodies that neutralize the adenoviral vectors limiting their transfection capacity.

### Lentiviral vectors

Lentiviruses belong to the Retroviridae family. The most commonly used lentiviral vector is based on the human immunodeficiency virus type 1 (HIV-1). Lentiviruses have the capacity to transduce non-dividing cells, a feature required for targeting cardiovascular tissue. They can carry genes up to 8 kilobase in size. The efficiency of myocardial transfection is similar to adenoviruses but with longer duration of gene expression.

Due to the derivation from HIV, lentiviruses raised biosafety concerns that were addressed by modifying the native vector. The modifications include deletion of all the wild-type HIV accessory proteins and modification of the
Table 1 Comparison of major viral vector systems.

<table>
<thead>
<tr>
<th>Vectors</th>
<th>Adenovirus</th>
<th>AAV</th>
<th>Lentivirus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Functional titer (per mL)</td>
<td>Up to $10^{12}$ dsDNA</td>
<td>Up to $10^{10}$ ssDNA 4.8 kb Yes - chromosome 19 - wild type AAV No - recombinant AAV Pseudo-random</td>
<td></td>
</tr>
<tr>
<td>Genome</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Insert capacity</td>
<td>7 to 30 kb</td>
<td>7 kb</td>
<td>10 kb</td>
</tr>
<tr>
<td>Integration</td>
<td>No</td>
<td>Yes - chromosome</td>
<td>No</td>
</tr>
<tr>
<td>Pattern of transgene expression</td>
<td>Transient</td>
<td>Long-term</td>
<td>Long-term</td>
</tr>
<tr>
<td>Cell-cycle dependent transduction</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Host/vector interactions</td>
<td>Cytotoxic and immunogenic</td>
<td>Minimally immunogenic</td>
<td>Minimally immunogenic</td>
</tr>
<tr>
<td>Clinical trial approved</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
</tr>
</tbody>
</table>

long terminal repeat (LTR) sequences of the vector [5]. These adjustments render the manufactured lentiviruses unable to replicate and minimize the risk of gene expression through recombination of a wild-type LTR with the therapeutic vector.

**Adeno-associated virus vectors**

Adeno-associated viruses (AAVs) are single-stranded DNA agents, members of the Parvovirus family. They are non-pathogenic with minimal immunogenicity and sustained expression which might be related to their inability to infect antigen-presenting cells. AAVs enter cells by endocytosis after binding to specific cell surface receptors. They are capable of transfecting non-dividing cells similar to cardiomyocytes. There are 11 identified serotypes of AAV, of which AAV1, 6, 8 and particularly 9 have excellent tropism for the heart [6]. The recombinant AAVs (rAAVs) used for gene therapy persist as episomal DNA in target cells instead of inserting into the human genome. This should render the risk of insertional mutagenesis from rAAVs negligible [7]. On the other hand, one of the major disadvantages of AAVs is their limited genome packaging capacity of 4.7 kilobase. In addition, since AAVs have single-stranded DNA, they require synthesis of a complementary strand before transcription of the delivered gene can happen. Thus, the onset of gene expression is delayed and peak levels are attained between 2 and 4 weeks postmyocardial delivery [8]. Moreover, human exposure to AAV capsid through natural infection or vector administration can result in production of all subclasses of Immunoglobulin G antibodies [9]. Natural exposure to AAVs results in the presence of neutralizing antibodies (NAbs) against some serotypes in about 20–40% of the population. Those antibodies, together with those generated by the immune system in response to previous treatment with similar viral vectors limit the efficiency of gene therapy with AAVs [10].

Despite their relatively high cost of production, rAAVs are currently considered to be an agent of choice for myocardial gene therapy. To date, there have been more than 20 trials using AAV vectors including the first clinical trial of AAV1/SERCA2a in advanced heart failure, all of which found no unexpected safety concerns [11].

**Methods of vector delivery**

In addition to the major advances in engineering vehicles for myocardial gene therapy, the last two decades have also witnessed development of novel strategies for gene delivery that we will briefly discuss in this section. Catheterization has been used to target the myocardium by antegrade intracoronary injection, retrograde coronary vein infusion and by direct endomyocardial or pericardial injection. Surgical delivery to the myocardium or pericardium can also be used and might be the preferred route in certain clinical settings.

**Antegrade injection**

Antegrade injection in the coronary circulation is characterized by its capacity to homogeneously deliver the vector to the myocardium. Agents increasing permeability of the vascular bed such as substance P, histamine or vascular endothelial growth factor, have been successfully used to improve the transduction efficiency of this technique [12]. Percutaneous coronary venous blockade can also enhance gene delivery when performed simultaneously with antegrade coronary injection [13]. In addition, the V-focus system [14] is a recently developed percutaneous modality that separates the coronary and systemic circulations to establish a closed circuit between the coronary arteries and the coronary sinus. The procedure involves three percutaneous catheters, two of which to perfuse the right and left coronary arteries and a third catheter with a balloon to occlude the coronary sinus. The perfusate circulates through an extracorporeal membrane oxygenation system that provides adequate myocardial oxygen supply. The perfusate circulates through an extracorporeal membrane oxygenation system that provides adequate myocardial oxygen supply. The V-focus system delivers the vector almost exclusively to the myocardium, keeping in mind however that less than 10% of the coronary circulation flow continues to communicate with the cardiac chambers and the systemic circulation through the Thebesian veins.

**Retrograde injection**

Percutaneous retrograde delivery of vectors in coronary veins has been validated in large animal models and achieved gene expression comparable to antegrade delivery.
The pressure resulting from antegrade flow is a limiting factor that is eliminated by simultaneous brief blockade of the coronary artery flow. Knowing that coronary veins remain mostly disease-free, the retrograde route is particularly useful when antegrade delivery is limited by a diseased arterial coronary circulation.

**Direct injection**

Direct injection of the vector into the myocardium can be achieved surgically or percutaneously. It overcomes numerous potential drawbacks that can be encountered with the intravascular route including the first-pass effect of the liver and spleen, the effect of neutralizing antibodies, the T-cell response and the impermeability of the endothelial barrier. Surgical gene transfer can be performed through a subxiphisternal or transthoracic approach and can be an attractive approach when the myocardium is easily accessible as during cardiothoracic surgeries. Catheter-based needle endomyocardial injection can be performed with several types of catheters and under different guidance modalities including fluoroscopy, electromagnetism and 3D mapping systems [16,17]. Both the surgical and the percutaneous approaches have limited vector delivery secondary to the restricted area of injection and the leakage at the site of the myocardial injection [18].

**Pericardial delivery**

The pericardial sac is a closed space in close proximity to the myocardium and is accessible to both surgical and percutaneous delivery. Vectors in this space preferentially transduce the pericardial cells with minimal myocardial expression. Administration of collagenase and hyaluronidase along with the vector can disrupt the pericardial cellular lining and extracellular matrix which improves myocardial

<table>
<thead>
<tr>
<th>System</th>
<th>Gene</th>
<th>Mechanism</th>
<th>Outcome</th>
<th>Clinical trials</th>
</tr>
</thead>
<tbody>
<tr>
<td>β-adrenergic system</td>
<td>β2-AR</td>
<td>Increases adenyl cyclase activity</td>
<td>Positive inotropic effect</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td>GRK2</td>
<td>Abolishing GRK2 reverses agonist-dependent desensitization of β-ARs</td>
<td>Positive inotropic effect and improved ventricular remodeling (inconsistent results)</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td>AC VI</td>
<td>Full mechanism remains unclear Increases cAMP generating capacity Improves phosphorylation of cTnl Improves cytosolic Ca&lt;sup&gt;2+&lt;/sup&gt; regulation</td>
<td>Positive inotropic effect Improved ventricular remodeling Reduced apoptosis Positive inotropic effect Improved ventricular remodeling Decreased oxygen cost of LV contractility Decreased incidence of ischemic ventricular arrhythmias</td>
<td>No</td>
</tr>
<tr>
<td>Ca&lt;sup&gt;2+&lt;/sup&gt; cycling proteins</td>
<td>SERCA2a</td>
<td>PLN inhibition relieves inhibitory effect on SERCA2a expression</td>
<td>Improved systolic and diastolic function (inconsistent results)</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td>PLN</td>
<td>Inhibits PP1 leading to phosphorylation of PLN and increased SERCA2a activity</td>
<td>Positive inotropic effect Improves diastolic function Ameliorates ischemia/reperfusion-induced injury</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td>I-1</td>
<td>Enhances the activity of ryanodine receptors and SERCA2a</td>
<td>Positive inotropic effect Improved ventricular remodeling Anti-apoptotic and positive inotropic effect in ischemia/reperfusion Anti-apoptotic effect</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td>S100A1</td>
<td>Regulation of calcium handling and decreased endoplasmic reticulum stress</td>
<td>Anti-apoptotic effect</td>
<td>No</td>
</tr>
<tr>
<td>Cell death</td>
<td>Bcl-2</td>
<td>Enhances cell survival</td>
<td>Anti-apoptotic effect</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td>I-1, S100A1</td>
<td>Gi-mediated pathway</td>
<td>Anti-apoptotic effect</td>
<td>No</td>
</tr>
</tbody>
</table>

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transduction. This is however associated with a higher systemic contamination and local inflammatory changes [19]. In addition to the surgical techniques, a percutaneous approach allows a safe and effective pericardial access appropriate for the delivery of therapeutic agents [20].

The optimal method of delivery depends largely on the proportion of target tissue to be genetically modified. Focal transduction might be desired to salvage an ischemic area whereas diffuse gene expression is more likely to reverse global myocardial dysfunction.

Heart failure gene therapy targets

The last 20 years witnessed significant evolution in our understanding of the pathophysiology of heart failure in its molecular and cellular dimensions which broadened the scope of interventions available for gene therapy. Far from being complete, we will discuss in this part some of the most important systems targeted to restore the function of failing cardiomyocytes. In Table 2, we list those systems along with their corresponding genes and therapeutic effects.

Targeting the β-adrenergic system

Cardiac beta-adrenergic receptors (β-AR) are mainly β1 subtype linked to a stimulatory heterotrimeric G protein (Gs) which stimulates adenylyl cyclase (AC) to produce a second messenger, cyclic adenosine monophosphate (cAMP). The latter acts through a series of intracellular signals involving a third messenger protein kinase A (PKA) which in turn phosphorylates a number of targets including troponin-I and L-type Ca²⁺ channels responsible for the physiologic effects of β-AR stimulation (Fig. 1). β2 receptors are also present, though to a lesser extent, in the heart. They are linked to Gs but also to an inhibitory G protein (Gi) which is responsible for inhibition of adenylyl cyclase. It is proposed that β2 receptors are more strongly coupled to Gs except in HF where their effect on Gi is increased. Chronic heart failure is associated with increased sympathetic outflow. Although this seems to be initially a compensatory mechanism, it is more deleterious in the long term. The β-adrenergic system is affected by multiple alterations including β-ARs downregulation, upregulation of β-ARs kinase and increased Gi function. These alterations lead to desensitization of the β-ARs and decreased signaling through their pathway.

Several gene-based experiments tested the hypothesis that genetic manipulation of the myocardial β-AR system can enhance cardiac function.

Overexpression of β-AR

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

Figure 1. Excitation-contraction signaling in cardiomyocytes with emphasis on targets for gene therapy. Interplay between calcium handling (purple) and β-adrenergic (blue) systems is illustrated. AC: adenylyl cyclase; ATP: adenosine triphosphate; β-AR: beta adrenergic receptor; I-1: (protein phosphatase) inhibitor-1; cAMP: cyclic adenosine monophosphate; Gs: stimulatory G protein; Gi: inhibitory G protein; NCX: sarcolemmal sodium/calcium exchanger; PKA: protein kinase A; PLN: phospholamban; PP: protein phosphatase; RyR: ryanodine receptor; SERCA: sarcoplasmic/endoplasmic reticulum calcium ATPase; SR: sarcoplasmic reticulum.
cardiomyopathy [21]. This finding reinforces the hypothesis that β-AR downregulation is a protective mechanism in the failing heart. In contrast, mice with cardiac overexpression of β2-AR demonstrated increased basal myocardial adenyl cyclase activity with increased left ventricular function [22]. Studying transgenic mice with different levels of β2-AR overexpression suggests that the heart tolerates enhanced contractile function via 60-fold β2-AR overexpression without detriment over more than 1 year and that higher levels of expression result in either aggressive or delayed fibrotic cardiomyopathy [23]. Based on those findings suggesting a potential benefit of β2-AR enhancement, adenovirus-mediated β2-AR gene delivery was studied in larger animals. Both direct and intracoronary myocardial delivery of adenovirus containing the human β2-AR transgene resulted in enhanced cardiac performance in rabbits [24,25].

Inhibition of G protein-coupled receptor kinases (GRKs)

The interaction between activated β-ARs and G proteins is regulated by kinases that modulate the receptor activity by phosphorylation of its carboxyl terminus. Agonist-dependent desensitization is mediated by a family of 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 [26]. There is controversy in the literature about the results of abolishing GRK2 activity. However, a recent study using 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 [27]. A peptide termed βARKct capable of inhibiting GRK2 mediated β-AR desensitization has been evaluated in vivo in animals. A study using intracoronary adenovirus-mediated βARKct transgene delivery to rabbits 3 weeks after induced myocardial infarction demonstrated a marked reversal of ventricular dysfunction [28].

Activation of cardiac AC expression

Although detrimental outcomes were demonstrated with multiple elements of the β-adrenergic system used to improve the expression of cAMP, activation of AC type VI (AC VI) seems to have a unique favorable profile. 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 [29]. This is in contrast with the steady increase in cAMP and the negative cardiac outcomes obtained after gene transfer of β-AR or Gs [30,31]. Increased expression of AC VI in a mouse model of acute myocardial infarction resulted in decreased mortality and preservation of LV contractility. In addition to improving cAMP generation, expression of AC VI in this particular study was associated with a favorable effect on Ca2+ handling [32]. 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 [33]. 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 [34].

Despite some controversy in the literature, there is a large volume of preclinical studies showing unequivocally that gene therapy targeting the β-AR system can be of therapeutic value in the treatment of HF. Until more studies and especially clinical trials take place, targeting the β-AR system is viewed as a promising field.

Targeting Ca2+ cycling proteins

Ca2+ plays a crucial role in contraction and relaxation phases of the cardiac cycle (Fig. 1). Ryanodine receptors (RyR) are proteins forming a link between the T tubules in the cardiomyocytes and the sarcoplasmic reticulum (SR). In a process known as “Ca2+ induced Ca2+ release”, depolarization activates voltage-operated L-type Ca2+ channels of the T tubule to allow Ca2+ entry into the cardiomyocyte. Ca2+ reaches the RyR protein modifying its molecular configuration which in turn opens the Ca2+ release channel of the SR releasing Ca2+ into the cytosol. This process greatly increases the concentration of Ca2+ in the cytosol allowing it to interact with troponin C triggering the contraction process. Relaxation occurs when Ca2+ detaches from troponin C and is either taken up by the SR via the action of sarcoplasmic/endoplasmic reticulum Ca2+-ATPase (SERCA) or extruded from the cardiac cell by the sarcolemmal Na+/Ca2+ exchanger (NCX). In humans, approximately 75% of Ca2+ is removed by SERCA. This protein is a Ca2+-ATP-dependent pump present in SR membrane and protruding into the cytosol. It exists in different isoforms, of which SERCA2a is the dominant cardiac form. This pump transports 2 Ca2+ across the SR membrane per each ATP used. In comparison, NCX removes one cytosolic Ca2+ per each ATP consumed (Na+-K+-ATPase uses 1 ATP to pump out three Na+ which have passively entered the cell in exchange for one Ca2+ via NCX) [35]. Phospholamban (PLN) is a protein implicated in regulation of SERCA2a activity. In its unphosphorylated form, PLN can inhibit SERCA2a. In contrast, phosphorylation of PLN relieves its inhibitory effect and results in increased SERCA2a activity with improved Ca2+ handling. Protein phosphatase 1 (PP1) is a type of serine/threonine phosphatase which has a major role in dephosphorylation of PLN in the heart [36]. In particular, stimulation of the β-adrenergic axis induces phosphorylation of a phosphatase inhibitor Protein-1 (I-1) resulting in PP1 inhibition and enhancement of myocardial contractile function [37]. HF is characterized by multiple defects in Ca2+-handling proteins with impaired SR Ca2+ loading and release. Reversal of those defects by gene therapy techniques has shown very promising results. We will review the main aspects of those novel therapies in this section.

Overexpression of SERCA2a

Consistently decreased SERCA2a activity has been identified in the failing human hearts [38]. Short-term improvement in cardiac contractility after gene transfer of SERCA2a has been demonstrated in HF rats [39]. 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 [40].

Decreased SERCA2a activity in HF is compensated by an increase in the extrusion of Ca2+ from the cardiomyocyte into the extracellular space by NCX. As mentioned above, NCX consumes twice the amount of energy required for uptake of the same amount of Ca2+ by SERCA2a [35]. Thereby, the altered ratio of SERCA2a/NCX activity in heart failure can contribute to a deleterious increase in energy consumption during excitation—contraction coupling. In rats with failing hearts secondary to diabetes mellitus, SERCA2a gene transfer was shown to decrease the oxygen expenditure for LV contractility to a normal level, in addition to restoring normal systolic and diastolic functions [39]. While β-adrenergic agonists improve contractility at the expense of increasing energy consumption and mortality, SERCA2a overexpression is a very promising approach to improve mortality by optimizing the mechano-energetic state of the heart.

Finally, a first-in-human clinical trial treating dilated cardiomyopathy with SERCA2a gene transfer is currently in progress and will be discussed in the last section of this review.

PLN inhibition

Another approach to improve Ca2+ handling involves inhibition of PLN. Inconsistent results were reported with PLN ablation ranging from deterioration of the cardiac function to restoration of myocardial contractility and Ca2+ homeostasis. Silencing of PLN expression in a sheep HF model resulted in improved SERCA activity along with improved systolic and diastolic LV function [41]. In addition, infecting human myocardial cells with an adenovirus encoding for an antisense of PLN showed an improvement in contraction and relaxation velocities similar to the benefit seen with adenoviral transfer of the SERCA2a gene [42]. In addition to the above conventional gene therapy strategies, RNAi therapy was used for the first time in a model of cardiac disease, specifically in rats with HF, in an attempt to suppress phospholamban expression. A rAAV-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 [43].

Active I-1 and Inhibition of PP1

HF is associated with elevated PP1 activity in humans resulting in dephosphorylation of PLN [44]. 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 [45]. Expression of a constitutively active I-1 in transgenic mice led to PP1 inhibition with increased phosphorylation of PLN and improved cardiac contractility [37]. 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 [46].

S100A1

S100 is a multigenic family of Ca2+-modulated proteins implicated in intracellular and extracellular regulatory activities. S100A1 is the most abundant S100 protein isofrom in the heart. It promotes cardiac contractile and relaxation function through enhancing the activity of both RyRs and SERCA2a. Loss of S100A1 in transgenic mice was associated with a reduced contractile response to acute β-adrenergic stimulation (isoproterenol injection), and a rapid deterioration of contractile function in response to chronic pressure overload by thoracic aortic constriction [47]. In a rat cryoinfarct model of HF, rAAV6-mediated long-term expression of S100A1 resulted in a sustained in vivo reversal of LV dysfunction and remodeling. In addition, this model showed additive beneficial effects when compared with and added to pharmaceutical β-AR blockade (using metoprolol), a feature of high clinical relevance suggesting that both treatments can eventually become additive in human heart failure [48].

Targeting cell death

Apoptosis is a process of programmed cell death that is involved in normal organ development. In a model of acute ischemia/reperfusion, overexpression of the anti-apoptotic protein Bcl-2, in transgenic mice reduced the rate of cardiomyocyte apoptosis and improved heart function [49]. Regulation of Ca2+ handling appears also as a potential pathway to limit apoptosis. As mentioned in a previous section, active I-1 expression in a mice model of ischemia/reperfusion improved Ca2+ handling and reduced apoptosis in association with suppressing markers of SR stress (caspase 12, Grp 78 and Inositol-requiring enzyme 1α) [46]. S100A1 was also reported to have an anti-apoptotic effect when added as an extracellular protein to a culture of neonatal ventricular cardiomyocytes [50]. On the other hand, the β-adrenergic system seems also to be an important modulator of apoptosis. Two different studies on rodent cardiac myocytes revealed that β2-AR stimulation reduces apoptosis via a Gi-mediated pathway, whereas β1-AR stimulation enhances apoptosis through a cAMP-dependent mechanism [51, 52].

Despite the early encouraging results in animal studies, anti-apoptotic strategies face multiple challenges before being considered for human trials. There remain some uncertainties about the reliability of the techniques currently used to estimate the rate of apoptotic cell death [53]. In addition, loss of the normal cellular regulation role provided by apoptosis can generate a serious risk of malignant cellular proliferation and autoimmune injuries. Finally, while ischemia/reperfusion injury appears to be amenable to intervention, it is less clear if other forms of cardiac injury and HF can benefit from anti-apoptotic strategies.

In summary, numerous molecular and cellular mechanisms incriminated in HF are currently within reach of gene therapy. The large amount of preclinical experience that we tried to summarize is an additional proof-of-concept that gene therapy, originally envisioned as a treatment for inherited monogenic diseases, can target acquired polygenic disorders such as HF. The expanding number of molecular targets and the innovations in gene-based therapies such as the recent use of the RNAi strategy are promising indicators
of the wide therapeutic scope and the high efficiency that gene therapy might potentially achieve. While the preclinical data will continue to expand, we are currently witnessing the birth of HF gene-based therapy in humans.

**Clinical trial**

After several years of preclinical experimentation, the first clinical trial of gene therapy in patients with HF was launched in the United States in 2007 [54]. Ca2+ upregulation 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. Cohorts 1, 2 and 3, of three patients each, received sequentially a single escalating dose of AAV1/SERCA2a. The infusion was spread over multiple coronary arteries in an attempt to provide homogeneous myocardial exposure. 6 to 12 month follow-up of these patients showed an acceptable safety profile. None of the serious adverse events reported was considered to be secondary to the study drug. Improvement was detected in several patients, reflected by symptomatic (five patients), functional (four patients), biomarker (two patients) and LV function/remodeling (six patients) parameters. In contrast to conventional inotropic agents, the study drug was associated with improvement in VO2 max which according to the authors may reflect the effect of SERCA2a on restoring an optimal mechano-energetic state of the heart. Although this is a phase 1 study involving a small number of patients, early results found that AAV1/SERCA2a treatment conferred qualitative biological benefit in every patient without pre-existing Nabs. This strongly supported the initiation of phase 2 of the trial which is a randomized, double-blind, placebo controlled, parallel-group, and dose ranging trial. Participants are currently being recruited and the estimated completion date of this phase is January 2010.

The results of this trial, in addition to larger trials of the same type, are certainly eagerly-awaited and will hopefully provide the scientific foundation to support gene therapy as a treatment modality for HF in humans.

**Conflict of interest statement**

Dr Roger Hajjar is scientific co-founder of Celladon, where he has major interest.

**References**


