Published in final edited form as:

Can J Cardiol. 2011 ; 27(3): 265–283. doi:10.1016/j.cjca.2011.02.005.

Targeted Gene Therapy for the Treatment of Heart Failure

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Abstract

Chronic heart failure is one of the leading causes of morbidity and mortality in Western countries and is a major financial burden to the health care system. Pharmacologic treatment and implanting devices are the predominant therapeutic approaches. They improve survival and have offered significant improvement in patient quality of life, but they fall short of producing an authentic remedy. Cardiac gene therapy, the introduction of genetic material to the heart, offers great promise in filling this void. In-depth knowledge of the underlying mechanisms of heart failure is, obviously, a prerequisite to achieve this aim. Extensive research in the past decades, supported by numerous methodological breakthroughs, such as transgenic animal model development, has led to a better understanding of the cardiovascular diseases and, inadvertently, to the identification of several candidate genes. Of the genes that can be targeted for gene transfer, calcium cycling proteins are prominent, as abnormalities in calcium handling are key determinants of heart failure. A major impediment, however, has been the development of a safe, yet efficient, delivery system. Nonviral vectors have been used extensively in clinical trials, but they fail to produce significant gene expression. Viral vectors, especially adenoviral, on the other hand, can produce high levels of expression, at the expense of safety. Adeno-associated viral vectors have emerged in recent years as promising myocardial gene delivery vehicles. They can sustain gene expression at a therapeutic level and maintain it over extended periods of time, even for years, and, most important, without a safety risk.

Chronic heart failure (HF), or the inability of the heart to sustain sufficient blood flow to meet the metabolic demands of the body, is the end-stage of the majority of cardiovascular diseases and is one of the leading causes of morbidity and mortality in Western countries.¹ Pharmacological approaches, such as β -adrenergic blockage, inhibition of the renin-angiotensin-aldosterone system, and diuretics, have managed to decrease morbidity and mortality in the past years. Nonetheless, the result is a regression in the disease progression, rather than reversal to a healthy state.^{2,3} New clinical trials targeting novel pathways, such as endothelin antagonists, vasopressin antagonists, and anti-tumour necrosis factor agents, have not produced strong support as to their therapeutic potential in extending the life span.³ Consequently, despite significant advances in drug discovery, the levels of morbidity and mortality remain inaptly high.¹ Chronic HF is still associated with increasing hospitalization costs and is a major financial load to the health care system.⁴ An additional ramification in

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Disclosures

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the remedy for HF is that its prevalence is growing concurrently with aging of the population, and advanced age is often a contraindication in terms of eligibility for heart transplantation despite some evidence to the contrary.^{5,6} Most important, heart transplant availability cannot meet the increased demand.^{1,7} All the aforementioned factors necessitate the exploration of novel therapeutic approaches, which will further the progress already achieved. Of the novel therapies that are being explored, cardiac gene therapy holds great promise.

Gene therapy is defined as the introduction of genetic material to cells or tissues with the purpose of treating a disease. The introduced genes can provide novel functions to the cells, improve or modulate existing ones, or replace deleterious mutants with functional ones. The introduction can be permanent, with the insertion of the DNA molecule into the cell genome, or transient to different levels, through the preservation of the DNA in an episomal state.⁸⁻¹⁰ Development of gene therapy in the early stages has been hampered by the lack of understanding of the disease. Methodologic breakthroughs, such as the development of transgenic animal models, have provided the means for the achievement of necessary proficiency and indepth knowledge in the cardiovascular research field.^{11,12} Candidate genes have been and continue to be identified. Calcium handling proteins are prominently targeted in gene therapy.^{8,13} Furthermore, sarcomeric and cytoskeletal proteins, as well as genes involved in cardiac signalling pathways, emerge as potent targets.⁸ This growing knowledge of the underlying mechanisms in the cardiovascular diseases in the past decades has lead to an inexorable and much needed march of progress in the field of cardiac gene therapy. The first gene therapy human clinical trial was performed in 1990 in patients with melanoma by introducing the neomycin resistance gene into tumour-infiltrating lymphocytes using a retroviral vector.¹⁴ The first trial against cardiovascular diseases was performed several years later by Isner et al.¹⁵ using direct naked DNA injection of the *VEGF* gene to treat hindlimb ischemia, whereas the first clinical trial directed to the myocardium for the treatment of coronary disease was performed 2 years later.¹⁶ Of all the clinical trials worldwide, 8.7% have targeted cardiovascular diseases. The vast majority has been approved in the United States, followed by the United Kingdom, Germany, and Canada.¹⁷

Cardiac gene therapy should ideally deliver the appropriate genetic material to a specific target cell/organ and achieve such expression levels to have a therapeutic effect for a defined duration of time, whether this is short or long term.

In the current article, we will review the field of myocardial gene therapy with emphasis on the vectors currently being used and the genes being targeted. We will focus our discussion on studies closely related to the myocardium, even though other tissues and cell types can also be targeted in cardiac gene therapy.

Cardiac gene delivery vectors

An important goal of gene therapy research is establishing an optimal gene delivery system. This should be safe, restrict expression to the desired cell/tissue, and provide ample expression levels. Vectors, the gene delivery vehicles, can be separated into two major categories: viral and nonviral. Both are currently being used in clinical trials at similar

levels.¹⁷ Although they all possess some of the desired features, one system that satisfies all criteria has yet to be discovered (Table 1). Even though nonviral gene delivery systems are still used in > 50% of clinical trials, research has been focused on developing viral systems, mainly due to the insufficient expression levels supported by the former.¹⁸ Adeno-associated viral (AAV) vectors have gained a lot of interest in recent years, mainly due to their low immunogenicity, their relative tropism, and their ability to sustain long-term expression without integrating into the host genome.^{9,18} This review will focus primarily on AAV-based viral systems, whereas other systems, such as adenoviral and lentiviral vectors, will only be briefly discussed.

Nonviral vectors

Nonviral vectors consist of plasmid DNA or small nucleic acids (antisense oligonucleotides, small interfering RNAs [RNAi]) that are delivered to target tissues either directly in their naked form or using some physical or chemical method to facilitate delivery.^{19,20} Naked DNA is the most fundamental, simple form of gene transfer. Its main advantage is the low immune response it elicits, which allows for readministration. Nonetheless, bacterial DNA has been known to be recognized by the immune system. To evade this immune response, research has been focused on developing mini-circle vectors that lack these sequences.^{21,22} A major advantage is that there are no size limitations, as with viral vectors, which allows the utilization of large transgenes or necessary elements in the expression cassette.^{18,23} Additionally, naked DNA can be produced in large amounts using standard techniques without any significant increase in cost, although significant improvements are constantly investigated,²⁴ is very stable and can be stored for prolonged periods of time without any effect on quality. The method of administration for naked DNA is direct injection, because DNA cannot cross the vascular cell barrier via infusion.^{25,26} The main disadvantage, though, is low transgene expression levels due to inefficient delivery and/or the transient nature of gene transfer, since it does not integrate into the genome and is degraded by the host cell.^{8,20,27,28} Despite the low transduction efficiency achieved by nonviral systems, they are still the vector of choice for > 50% of clinical trials, mainly by virtue of their safety.¹⁷

Viral vectors

Viruses, most of which are pathogenic, are infectious agents that have the innate dexterity to efficiently invade host cells and use the host gene expression machinery to replicate. Viral vectors were designed to take advantage of this ability to deliver and express therapeutic genes in target cells, and this is their main advantage over the nonviral vectors, which result in low efficiency of gene transfer. All or most of the viral genome is replaced by the expression cassette of choice, concurrently rendering the virus nonpathogenic. Viral vectors can be divided into integrating and nonintegrating. Three types of viral vectors are profusely used in gene therapy for cardiovascular diseases: lentiviral, adenoviral, and AAV vectors. Of these, lentiviral and AAV vectors are potentially integrating, whereas adenoviral vector DNA remains episomal.^{8,9} Lentiviral and adenoviral vectors were the first to be used, but AAVs have gained a great deal of interest in the past years, mainly thanks to their advantages regarding safety.

There are several attributes of the gene therapy viral vectors that should be deliberated on and will be discussed in each section: (1) gene transfer efficiency and duration of expression, (2) tissue tropism, and (3) safety—potential immune and inflammatory response.

Adenoviral vectors

Adenoviruses were first isolated > 50 years ago from adenoid tissue. They are involved mostly in upper respiratory infections. They are nonenveloped viruses, have a moderate size of 60–90 nm, and contain a linear double-stranded DNA genome, which remains episomal after infection.²⁹ They bind to the cellular receptor CAR (coxsackievirus and adenovirus receptor), which is expressed in cardiomyocytes but also in liver and the respiratory tract.^{30–32} Adenoviral vectors can efficiently transduce both dividing and nondividing cells. They have high expression kinetics leading to expression only 2–3 days after injection in experimental animals.^{33–35} Furthermore, their episomal nature eliminates the risk of insertional mutagenesis or oncogenesis.³²

Replication-deficient adenoviral vectors, stemming from serotypes 2 and 5, are the most popular viral vectors for clinical gene therapy for cardiovascular diseases so far, mainly owing to their transduction efficiency.^{17,29,36} Major concerns, however, about the safety of adenoviral vectors were raised after the death of Jesse Gelsinger in 1999 due to a massive immune response following the injection of an adenoviral vector.^{37,38} Considering that adenovirus is a pathogen causing the common cold, it is expected that several individuals will have neutralizing antibodies against the capsid proteins, which results in the inactivation of the vector. Most important, it has been known that viral capsid proteins can stimulate innate and adaptive immune responses and cause inflammation, leading to the elimination of the adenoviral vector and the host cells expressing the transgene.^{39–42} To quench the immune response,^{43,44} vectors that lack some or all (gutless) viral genes were developed.⁴⁵ It is noteworthy, however, that even the “gutless” vectors induce immune responses against the capsid, although the duration of expression was still prolonged.^{46–48}

Lentiviral vectors

Lentiviruses have been used in the past few years as vectors for cardiac gene transfer. They are a subclass of retroviruses, whose name stems from the Latin word *lenti*-, which means “slow.” Their major advantage is that they can stably infect nondividing cells, such as the terminally differentiated cardiac myocytes.⁸ Lentiviral vectors are based primarily on human immunodeficiency virus (HIV), which is a known pathogen,⁴⁹ hence raising safety concerns regarding wild-type contaminations in the viral preparations. Moreover, HIV exhibits a natural restricted tropism for CD4⁺ cells, which is of course undesirable. Finally, production yields are hampered with low efficiency. New technologies, however, are developed to overcome these hurdles.^{8,50–55} The major limitation of lentiviral vectors is the risk for insertional mutagenesis, as seen with other retroviral vectors. Several strategies are used to improve safety and ascertain the episomal state in the host cell,⁵⁵ but it is noteworthy that lack of integrase activity in these vectors results in reduced duration of expression in a rat model of myocardial gene transfer.⁵⁶ The application of lentiviral vectors in the cardiovascular gene therapy field mostly pertains to ex vivo applications. Namely, they are used to stably transduce cells, mostly embryonic stem cells.^{57–59} Lentiviral vectors have also

been used in vivo, for cardiac allograft transduction,^{60–62} as well as for direct cardiac gene therapy. Successful gene transfer has been established, although these vectors fail to consistently maintain long-term expression,^{63–65} such as the one seen with recombinant AAVs (rAAVs), without integrating into the host genome.^{56,66,67}

Adeno-Associated viral vectors

AAV was discovered > 40 years ago as contaminant in a preparation of Simian adenovirus. AAVs were considered defective viruses, as they could not replicate without the presence of adenovirus.^{68,69} They are small viruses, approximately 25 nm in diameter, that infect humans but are not pathogenic—namely, they are not so far been associated with a disease.^{70,71} They carry a single-stranded DNA genome and belong to the Parvoviridae family, the *Dependovirus* genus.^{72,73} So far, at least 13 naturally occurring serotypes and > 100 variants have been identified.^{18,68,74–83} rAAV vectors are generated by replacing the 4.7-kb size viral genome with the expression cassette of choice, flanked by the viral ITRs.^{74,84–86}

rAAVs as vectors for gene therapy have gained a lot of attention in the past 3 decades, following the establishment of the first rAAV production in a seminal study by Samulski et al.⁸⁷ In the 1990s, several human clinical trials used rAAV2, but in the last decade there has been a significant increase. Seventy-five clinical trials have been at least initiated, if not completed, using AAVs so far, starting as early as 1994, which accounts for 4.5% of all trials.¹⁷ This increasing interest in rAAVs in the past years is mainly due to their unique advantages as gene therapy vectors. They are relatively safe, as they are nonpathogenic viruses and they are not immunogenic. This means that the inflammatory response toward these vectors is minimal, especially compared to adenoviral vectors.^{39,40} They can infect both dividing and nondividing cells and maintain long-term gene expression. Once in the nucleus of the host cell, rAAV genomes undergo concatamerization and partial circularization (Fig. 1).^{83,89} This genomic structure is maintained for long periods and is associated with the extended expression duration.^{83,90} The fact that they remain episomal eliminates the risk of insertional mutagenesis, although under rare circumstances it has been shown that the AAV genome can integrate.⁹¹ A key advantage of rAAVs is that even though their genome remains episomal, they can still drive long-term expression. In several clinical trials, sustained transgene expression has been observed for 6 months to 3.7 years in patients^{92–95} and up to 8 years in primates and canine models.^{96,97}

Another important advantage of rAAVs is their wide, yet distinct, tropism of each serotype.^{74,81,98–106} Among the AAV serotypes, AAV1, AAV6, AAV8, and AAV9 have been identified as the most cardiotropic,^{107,108} with serotypes 1 and 6 already validated in human clinical trials.¹⁷ Interestingly, their tissue tropism seems to alternate depending on the species, from small to large animals, which naturally raises caution when translating research findings.^{108–121} Overall, in smaller animal models, the cardiotropic serotypes show preference for the myocardium, but they are not restrictive. In larger animals, the cardiac-targeted delivery methods^{119,121} preclude a definite answer as to their specificity toward cardiac tissue, although that seems likely.^{115,121} In the past few years, transductional and transcriptional targeting has been explored with varying degrees of success, to restrict expression only to the myocardium.^{111,122–134}

Several additional limitations of rAAVs include the small size of insert they can accommodate, the slow kinetics, and levels of gene expression, although new technologies have developed to overcome these hindrances.^{11,135–140} Finally, the risk for insertional mutagenesis stems mainly from the ability of wild-type AAVs to integrate in a specific site in chromosome 19 in humans, termed AAVS1.¹⁴¹ Most studies argue the episomal state of rAAV genomes in the host nucleus.^{99,141} Recent studies, however, suggest the occurrence at low frequency of such integration events in tissues such as cardiac and skeletal muscle,¹⁴² although the induction tumourigenesis has been disputed.^{91,143–145}

rAAV vectors have been favored, among other things, based on their low immunogenicity. Compared to adenoviral vectors, the inflammation subsequent to gene transfer is low and highly transient, without immunotoxicity toward the target organ.^{39,41} Several studies over the past decade, however, point to the existence of immunity, preexisting and acquired, against AAVs. Considering that most of the serotypes were identified as integrated proviral sequences from human tissues and that humans are natural hosts for AAVs,^{39,83} it comes as no surprise that humans have preexisting antibodies against several serotypes.¹⁴⁶ In a worldwide epidemiologic study, it was found that AAV2 exhibits increased seroprevalence,¹⁴⁷ especially compared to other serotypes, such as AAV1, AAV7, and AAV8.¹⁴⁶ AAV serotypes 5, 8, and 9 exhibit the lowest seroprevalence in healthy populations.¹⁴⁸ It is self-evident that the presence of preexisting antibodies greatly impedes the gene transfer efficiency and should be taken into consideration when determining eligibility to participate in a clinical trial. Of course the route of administration is critical in this matter.³⁹ The immune response against AAVs is, as mentioned, low and transient. Nonetheless, it can lead to clearance of the transgene expressing cells, through possibly the expansion of preexisting memory CD8⁺ T cells.^{149–151} It is noteworthy that the AAV capsid has been implicated in these responses.^{152,153} Other studies, however, suggest that this T-cell response is not cytolytic in vivo.^{154,155} These discrepancies are indicative of an elementary complication in clinical trials: the difficulty to translate outcomes from preclinical studies. Research in this field in the past years has revealed, if nothing else, that the immune system in murine models is quite different from that in humans, making it difficult to predict or explain the results of clinical trials.^{156,157} With immune responses to AAV vectors a stark reality, several strategies have been explored to circumvent this problem. Transgene expression only in the desired tissue and not in antigen-presenting cells (APCs) has been shown to limit the extent of immune reaction. Additionally, the temporary use of immunosuppressive drugs, such as cyclosporine or LEA29Y, has been suggested. It is of note that long-term expression can only be achieved by the induction of tolerance to the transgene, which can be prevented by strong immune suppression.^{157,158} Considering that the immune response is designed against naturally occurring serotypes, several strategies have aimed at modifying the capsid itself.¹⁵⁹ Furthermore, different serotypes produce varying degrees of response in different individuals, hence allowing for serotype switching strategies.¹⁵⁷ Overall, immune response to AAV vectors appears to be a major impediment to clinical trials. Nonetheless, novel strategies to evade immune responses, such as modulating vector dose, capsid tailoring, immune suppression, induction of tolerance by specific organ targeting, and transcriptional control of expression, offer great hope.¹⁶⁰

Gene delivery methods

Gene therapy vectors have to overcome several physical barriers to reach the target cells. Cardiotropic vectors are indispensable for gene therapy applications, mainly because they ascertain safety by virtue of excluding nontarget tissues. Nonetheless, they, too, have to surpass several obstructions to deliver genes to the target tissue. Viral vectors in particular have to overcome neutralizing antibodies in the bloodstream, an interaction that, immediate inactivation notwithstanding, can cause downstream immune activation and a detrimental inflammatory response. A second important barrier is the endothelial cell lining, which has been shown to hinder viral gene transfer in several studies.^{98,139,161,162} Last but not least, once the delivery vehicle is in the interstitial space, it has to scavenge naturally occurring endocytosis and intracellular trafficking pathways and release the genetic material to the nucleus for expression.⁸⁸ Consequently, an optimal gene delivery method, one that assists the gene vehicle to overcome these barriers, is imperative for successful therapy. Clinical cardiac gene therapy poses tremendous challenges, as the simplified intravenous delivery used in small animals can lead to diffusion of the vector. There are some general criteria by which a given delivery method can be validated: the overall transductional efficiency, the distribution of transduction, the “leakage” of vector to nontarget tissues and their subsequent transduction, the technical difficulty of the performed operation (mainly associated with the level of invasiveness), and, finally, the complications stemming from the process, such as an inflammatory response due to injuries.¹⁰ Cardiac gene delivery techniques can be classified into two major categories: direct injection and perfusion/infusion. The major distinction between the two is that the former circumvents at least one barrier, the endothelial capillary layer.

Direct injection has been predominantly used for nonviral vectors, due mainly to their inability to efficiently cross the endothelial cell layer. Furthermore, naked DNA has no inherent transductional tropism, which necessitates its delivery as close to the target cell as possible.^{10,19} Intramyocardial injection of naked DNA in both murine and porcine animal models carrying a reporter gene has been shown to lead to transgene expression, albeit locally. The expression pattern is patchy and usually does not extend more than a few millimetres around the injection site.^{10,163–166} Adenoviral and rAAV vectors have also been delivered using this technique.^{163,165,166} To achieve a clinically pertinent broader delivery, multiple injections around the “target” area in the myocardium are usually performed.^{167,168} Intramyocardial gene delivery through the epicardium can be performed during thoracotomy, which is highly invasive, with all the accompanied risks of a surgical procedure. Catheter-based intramyocardial gene transfer through the endocardium is a less invasive alternative, although the target myocardial layer is different.^{167,169} It is of note that despite the localized nature of this delivery method, noncardiac tissues, such as liver and lung, were also transduced.¹⁶⁷ A major issue with intramyocardial injections is the induction of an acute inflammatory response, which stems from the injury caused by the injection, rather than the viral vehicle itself, as it is also observed in saline-injected animals.^{170,171} Despite these hindrances, intramyocardial injection is still the delivery of choice in human clinical trials where naked DNA is used (Euroinject One clinical trial).^{172–174} Intrapericardial gene delivery has also been explored as it provides the means for a more homogeneous

distribution of the gene vector, without the side effects of intramyocardial injection injury. This method results in limited transgene expression, confined to an outer layer of the epicardium, the width of a few cells.^{175,176} To improve the low penetration levels, in a rat model, a mixture of collagenase and hyaluronidase was used together with the viral vector. This led to an increase in the diffusion that reached 40% of the myocardium.¹⁷⁷ Of course, the use of such enzymes would pose safety concerns in human clinical trials.

The main advantage of gene delivery by direct injection is the evasion of the capillary barrier. On the other hand, the limited spacial myocardial distribution is a major impediment. An alternative approach is transvascular gene transfer, where the limitation becomes an advantage and vice versa. Injection of gene vectors into the coronary circulation can ascertain a homogeneous expression in a relatively defined target area.^{178,179} In small animal models, such as rat, it has been shown that increasing the vector dwelling time in the coronary vessels by cross-clamping the aorta and pulmonary artery can significantly increase transduction efficiency.^{180–182} In mice, this technique is combined with lowering of the body temperature and cardioplegia.^{139,161} Cross-clamping, however, is not feasible in larger animals and particularly in failing human hearts.¹⁰ In larger animals, as well as in humans, in order to achieve prolonged exposure of the vasculature to the vector and allow for the crossing to take place, cardiopulmonary bypass (CPB) is applied. CPB gene transfer offers several advantages: (1) provision of increased dwelling time, (2) removal of blood cells from the circulation, (3) concordantly called crystalloid cardioplegia, the possibility to apply lower temperatures, and (4) the option of recirculation of the gene delivery solution through the coronary system.^{183–186} Due to the invasiveness of the CPB procedure and its morbidity risks, its use for gene delivery in humans is indicated for patients already undergoing CBP.

Intracoronary gene delivery, in large animals and humans, can be performed either retrograde or antegrade. Both delivery methods are catheter based, which entails the obvious advantage of a minimally invasive percutaneous procedure. Percutaneous selective pressure-regulated retroinfusion of the coronary veins in pigs has been found to permit extended dwelling time, which allows for the gene delivery vehicle to attach to the capillary wall and cross the endothelial barrier. It has been shown to be more efficient, in terms of transduction levels and distribution profile, than direct intramyocardial injection.¹⁸⁷ This technique can overcome the hindrances posed by occluded or stenotic coronary arteries, which is naturally of clinical significance.¹⁸⁸ The enhanced transduction stems mainly from the efficient filtration of the vector from the capillaries to the interstitial space due to the application of higher pressure gradient, as the filtration takes place on the venous side of the arteriolar resistor.^{10,183}

Catheter-based percutaneous antegrade coronary myocardial gene transfer is the most pertinent to human gene therapy applications, especially because it provides a high, homogeneous transduction.^{189,190} Antegrade coronary gene delivery has already been studied in large animals and satisfies these criteria, especially after the development of several optimization modus operandi. It has been shown in piglets that application of balloon intraluminal seal and a slow infusion flow rate are important if safety and efficiency are to be met. In particular, the slow infusion rate was shown to decrease myocardial injury and the

intraluminal seal is necessary to prevent washout of the vector and subsequently collateral transduction of distant, nontarget organs.¹⁹¹ In a swine large animal model, it was shown that with the use of standard techniques of percutaneous catheterization, it is possible to achieve high cardiac transfection efficiency. Indeed, the percutaneous antegrade epicardial coronary artery infusion cardiac gene delivery method has already been used in a human clinical trial. The observed improvement in a small cohort of patients was significant to justify the commencement of phase 2 in this trial. It is of note that coronary occlusion through the use of the V-focus recirculation device has also been used effectively in large animals.^{10,92,192}

Finally, aside from the physical optimization techniques to improve perfusion-based myocardial gene transfer, several chemical compounds have been used toward the same objective. As mentioned, the perfusion methods have to overcome the capillary wall barrier to reach the interstitial space. Capillary modulating substances commonly used are vascular endothelial growth factor (VEGF), sildenafil, histamine, serotonin, adenosine, nitroprusside nitroglycerin, and heparin.^{92,117,139,161,181,193–195} VEGF is the most commonly used microvascular permeabilizing agent, which exerts its effect through nitric oxide (NO).^{117,194,196} The combination of VEGF with sildenafil, to potentiate the VEGF effect, was even more beneficial.¹⁷⁸ Nitroprusside and acetylcholine have been used successfully in rodents,¹⁹⁷ whereas adenosine has been used in larger animals.^{166,181} Nitroglycerin has also been used extensively in larger animal models and clinical trials.^{92,178} Overall, catheter-based intra-coronary infusion systems satisfy the criteria for homogeneous, highly efficient, and safe cardiac gene transduction. However, further interventions, physical and pharmacological, need to be explored to attain optimal, clinically relevant cardiac gene delivery.

Gene Targets

Angiogenic gene therapy

Angiogenesis is the physiological process of developing new blood vessels from preexisting ones, or the process by which preexisting vessels expand, lengthen, or sprout. It can be both physiological and pathologic. Angiogenesis is induced by several angiogenic factors. The use of angiogenic factors is prominent among gene targets in human clinical trials for cardiovascular diseases.¹⁷ The most conspicuous ones are VEGF, FGF (fibroblast growth factor), HGF (hepatocyte growth factor), and PDGF (platelet-derived growth factor).¹⁹⁸

Five VEGF genes have been identified and 5 VEGF-A isoforms.¹⁹⁹ V plasmid gene transfer was used for the first human clinical trial for cardiovascular diseases by Isner et al.¹⁵ VEGF induces both angiogenesis and vasculogenesis, namely the de novo formation of vessels during development.^{200,201} Research over the past 2 decades has revealed a more complex role; it has a vasodilatation and a hypotension effect. It is also involved in the maintenance of vascular homeostasis and structure and adaptive cardiac growth. Interestingly, VEGF has an endothelial specific autocrine and paracrine signalling, which cannot be compensated with exogenously supplied VEGF. It is of note, however, that angiogenesis has been linked to atherosclerosis in animal models, although, overall, research studies regarding this effect suggest that neovascularization is a parallel, rather than causative, phenomenon (for a

comprehensive review, refer to Zachary and Morgan²⁰² and Khurana et al.²⁰³). It is self-evident that VEGF, by virtue of angiogenesis induction, has therapeutic potential for ischemic heart disease, an effect already observed in animal models.²⁰² In humans, it has been used for the treatment of 2 diseases: ischemic peripheral artery disease and coronary artery disease, with the aim of generating collateral blood supply that circumvents the occluded artery.¹⁹⁸ Overall, > 50% of the clinical trials for cardiovascular disease are aimed at inducing angiogenesis using growth factors, mainly VEGF.^{17,202,204} More than 20% of them are phase II or phase III.¹⁷ For the coronary artery disease clinical trials, VEGF165 has been delivered using naked plasmid, liposomes, or adenoviral vectors.^{174,205,206} Adenoviral gene transfer in the Kuopio Angiogenesis Trial (KAT)²⁰⁵ resulted in significantly increased cardiac perfusion, and after an 8-year follow-up, it was deemed safe, as there were no side effects, such as arrhythmias or cancer.²⁰⁷ The EuroInject One clinical trial also found that VEGF165 administration resulted in increased perfusion.¹⁷³ In the REVASC clinical trial, where AdVEGF₁₂₁ was delivered by intramyocardial injection, exercise duration and angina symptoms were improved.²⁰⁸ The NOGA angiogenesis Revascularization Therapy: assessment by RadioNuclide imaging (NORTHERN) trial in which 93 patients with class 3 or 4 angina received intramyocardial injection of a plasmid carrying the VEGF165 gene. The patients who received the treatment, however, did not show improvement in several parameters, such as treadmill exercise and angina symptoms.²⁰⁹ A known side effect of VEGF is dose-dependent peripheral edema through induction of increased permeability of blood vessels via NO signalling.^{196,210} This side effect has been observed in clinical trials, such as the RAVE trial.²¹¹ Additionally, VEGF has been implicated in cancer, but cancer has not been observed in any clinical trials.^{202,203,207}

FGF is another angiogenic factor, which is used alone or in combination with VEGF, as it has been suggested that it promotes VEGF expression, which is a prerequisite for the angiogenic effect. The FGF family has the most versatile signalling functions and several growth factors are downstream of that signalling.²¹² Concordantly, interpretation or prediction of clinical outcomes becomes very complicated. Of this family, 2 isoforms have been used in clinical trials: FGF2 and FGF4. FGF2 or basic FGF (bFGF) is the one shown to have the most angiogenic potential in preclinical studies.²⁰⁴ FGF2 using recombinant protein in clinical trials failed, however, to show significant benefits.²¹³ FGF4 is a secreted factor that offers the added advantage of paracrine effect. Adenoviral gene transfer of FGF4 in the AGENT (Angiogenic Gene Therapy) phase I clinical trials resulted in improvements in exercise duration and angina symptoms, but the phase III trial failed to show similar results.^{214,215} These trials revealed a beneficial effect for women participants, which led to the AWARE (Angiogenesis in Women with Angina pectoris who are not candidates for REvascularisation) clinical trial.^{202,214} The most promising results for FGFs stems from the TALISMAN 201 (Therapeutic Angiogenesis Leg Ischemia Study) trial. In that trial, *FGF1* plasmid gene delivery resulted in 39% reduction of major amputation risk.²¹⁶

HGF is synthesized in the liver and has been demonstrated to exert regenerative and antifibrotic effects. Several animal studies have shown angiogenic properties. A human phase I/II clinical trial, the TREAT-HGF (Japan Trial to Treat Peripheral Arterial Disease by Therapeutic Angiogenesis Using HGF Gene Transfer), has shown promising results in the treatment of critical limb ischemia (CLI).^{204,217}

Overall, angiogenic gene therapy shows great potential for the treatment of ischemic cardiac disease. However, further studies of the underlying mechanisms and the development of novel, more efficient, gene delivery systems is warranted. Additionally, careful forethought should be given to the hypervascularization risk originating from induction of angiogenesis for a prolonged period of time. Consequently, gene delivery vectors that produce transient gene expression might be more suitable for angiogenic gene therapy.

Targeting Ca^{2+} cycling proteins

Calcium is a critical mediator of excitation-contraction coupling in cardiac cells. Action potential at the cell membrane induces a minor Ca^{2+} -influx through sarcolemmal L-type calcium channel (LTCC) (Fig. 2). This triggers further calcium release from the sarcoplasmic reticulum (SR) via the major SR Ca^{2+} -release channel ryanodine receptor (RyR2, the cardiac isoform of RyR). This calcium then binds to the myofilament protein, troponin C, resulting in sarcomere shortening and muscle contraction. Muscle relaxation is initiated by Ca^{2+} dissociation from the troponin complex and its reuptake into the SR by SERCA2a (75% of Ca^{2+} removal in humans) its removal by the sarcolemmal $\text{Na}^{2+}/\text{Ca}^{2+}$ exchanger (NCX) (25% of Ca^{2+} removal in humans). SERCA2a pumps serve a dual function: (1) to cause muscle relaxation by lowering cytosolic $[\text{Ca}^{2+}]$ 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+} . Ca^{2+} cycling is regulated by the β -adrenergic signalling system through phosphorylation and dephosphorylation (Fig. 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 RyR2, thus increasing cytosolic Ca^{2+} rise, 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 heart (Fig. 2).

Chronic HF is associated with significant abnormalities in calcium cycling, which are due to SR Ca^{2+} leak through the RyR2, decreased SR Ca^{2+} uptake, and decreased SR Ca^{2+} content. The importance of SERCA2a in HF has been studied extensively in both animal models and humans. These studies have shown that SERCA expression and activity are reduced in failing myocardium. Increased inhibitory function of PLN and increased activity of PP1 resulting in dephosphorylation and inactivation of PKA targets 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.

Overexpression of SERCA2a—A large number of studies have shown that restoration of SERCA2a by gene transfer in failing cardiac cells, rodent models, and large animals models of HF results in cardiac improvement. Gene transfer of SERCA2a to failing human

cardiomyocytes restored the calcium transient and improved contraction and relaxation velocity to the levels of nonfailing myocytes.²¹⁸ 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. Calcium overload leads to increased Ca^{2+} uptake by mitochondria, resulting in mitochondrial dysfunction and decrease in ATP production.^{219,220} Normalization of intracellular calcium by SERCA2a overexpression prevents mitochondrial dysfunction and decrease energy cost for Ca^{2+} regulation. 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 models of HF. Therefore, SERCA2a gene transfer restores cardiac energetics both by improving mechanoenergetic efficiency and by enhancing energy supply. Diastolic Ca^{2+} overload occurring in ischemia and chronic HF is responsible for cellular electric instability resulting in tachyarrhythmias.²²¹ 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 afterdepolarizations.²²² Recent data demonstrated that SR Ca^{2+} load via SERCA2a is responsible to sensitization of RyR, thereby creating a travelling wave of high Ca^{2+} sensitivity initiating Ca^{2+} release.²²³ SERCA2a overexpression in rabbit cardiomyocytes, favouring sequestration of Ca^{2+} in the SR, resulted in rapid inactivation of subsequent Ca^{2+} currents and reduced Ca^{2+} entry through LTCC.²²⁴ In this model, reduction of transsarcolemmal Ca^{2+} flux by SERCA2a overexpression without changes of normal systolic transient also reduced the occurrence of aftercontractions and afterdepolarization.²²⁴ As such, reduction of cytosolic Ca^{2+} overload might constitute a viable strategy for combating of arrhythmia. Several reports have also shown that SERCA2a overexpression rescues or prevents cardiac hypertrophy.^{225,226} 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 VSMC.²²⁷ In VSMC, SERCA2b (ubiquitous isoform) and SERCA2a are co-expressed in adulthood. During proliferation, VSMC undergoes dedifferentiation and acquisition of a synthetic phenotype, associated with loss of SERCA2a. Restoring SERCA2a expression in VSMC through gene transfer inhibits VSMC proliferation and postinjury restenosis in a rat carotid injury model.²²⁷ NO synthesis is controlled by intra-cellular Ca^{2+} oscillations, and increase in SERCA activity through overexpression of S100A1 (see later) also increases NO production in endothelial cells.²²⁸ Sakata et al.²²⁶ showed that transc coronary gene transfer of SERCA2a increases coronary blood flow. One of the reasons for this improvement is the increased production of NO caused by SERCA2a overexpression in endothelial cells.²²⁹ This additional effect of SERCA2a overexpression might have a synergistic benefit on cardiovascular disease. Overall, SERCA2a gene transfer has been shown to have a significant number of effects on the cardiovascular system beyond increasing contractility.

PLN inhibition—Another approach to improve Ca^{2+} 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 Ca^{2+} homeostasis. Silencing of PLN expression in a sheep HF model resulted in improved SERCA activity along with

improved systolic and diastolic left ventricular function.²³⁰ 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.³⁴ 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 PLN expression. An rAAV-RNAi vector generated stable cardiac production of a regulatory RNA sequence, which in turn suppressed PLN expression. SERCA2a protein was subsequently increased accompanied by restoration of systolic and diastolic cardiac function.²³¹

Active I-1 and inhibition of PP1—HF is associated with elevated PP1 activity in humans resulting in dephosphorylation of PLN.²³² 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.^{233,234} Expression of a constitutively active I-1 in transgenic mice led to PP1 inhibition with increased phosphorylation of PLN and improved cardiac contractility.^{234,235} 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 endoplasmic reticulum (ER) stress response.²³⁴

S100A1—S100 is a multigenic family of Ca^{2+} -modulated proteins implicated in intracellular and extracellular regulatory activities. S100A1 is the most abundant S100 protein isoform in the heart. It promotes cardiac contractile and relaxation function through enhancement of 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.²³⁶ In a rat cryoinfarct model of HF, rAAV6-mediated long-term expression of S100A1 resulted in a sustained in vivo reversal of left ventricular dysfunction and remodelling. In addition, this model showed additive beneficial effects 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 HF.²³⁷

Targeting the adrenergic system

The β -adrenergic system is desensitized in chronic HF. This desensitization emanates from the deregulation of key components of this signalling pathway, which include but are not limited to the β AR, AC, and G protein–coupled receptor kinase (GRK). Consequently, increased effort has been invested in identifying therapeutic strategies that regulate this system and its components.

Adrenergic receptors—ARs belong to the G protein-coupled receptor superfamily. There are several adrenergic receptors subtypes, but β -1 (β 1) and -2 (β 2) are the predominant forms in the heart. They are the physiological modulators of cardiac function in response to various stimuli.²³⁸ In pathologic conditions, where cardiac contractility is compromised, the

heart responds with activation of the neurohumoral system and β AR stimulation. Chronic β AR stimulation, however, has detrimental effects that spring mainly from its inactivation, which is widely accepted as a cardioprotective mechanism.²³⁹ The 2 receptors activate signalling pathways in divergent manner. β 1AR, the most abundant subtype (70%-80%), acts mainly on the AC-cAMP-PKA pathway.^{239,240} β 2AR acts on the same pathway but with less efficiency for the ventricles, and it has also been reported in rodents to be G_i coupled.^{239,241} In HF, these subtypes might actually confer different outcomes, with β 1AR inducing apoptotic signalling cascades and β 2AR inducing antiapoptotic ones.²³⁸ The desensitization of β ARs and the increase in the ratio of β 1: β 2AR are hallmarks of disease conditions. Overexpression of β AR has been sought as a straight forthright response to the desensitization. However, in several studies transgenic mice overexpressing β AR develop HF.²³⁹ Additionally, the use of β -blockers in patients with HF decreases mortality,^{239,242,243} and this direction is seen as the predominant future therapeutic approach using a variety of strategies.²³⁹ Interestingly, overexpression of β 2AR in some animal models results in improved cardiac function,^{244–246} although the effect is largely the same as that of β 1AR.
244,247–249

Inhibition of GRK—Seven members of the GRK family have been identified. Their main action is the phosphorylation of GPC receptors. They act as the “off-switch,” as phosphorylation of these receptors results in the uncoupling of bound G proteins, which act as effectors of downstream signalling pathways.^{238,250,251} Three GRK isoforms are expressed in the heart, with GRK2 being the predominant one and having been studied extensively in cardiovascular diseases. GRK expression is increased in the ventricles and considerably more in the atria of patients with HF of different origin.²⁵² This increase also correlates with the stage of HF. In end-stage HF, the levels decrease to those seen in control hearts.^{241,253} In animal models of HF, GRK2 overexpression is also expressed.²⁵⁴ Inhibition of GRK function could therefore provide a therapeutic target for HF. To this end, several research groups have investigated the effect of a peptide analog of GRK, the GRK2 C-terminus, called β ARKct. Transgenic overexpression, in vivo viral gene transfer, and in vitro gene transfer of β ARKct seem to ameliorate HF.^{250,254}

Adenylyl cyclase—The stimulation of β ARs leads to the conformation change of the receptor, the activation of G_s proteins, bound to the receptor, which then activate AC and this results in the production of cAMP. There are 9 isoforms of adenylyl cyclase. AC5 and AC6 are the predominant isoforms in the heart.^{239,255,256} ACs are downregulated in the failing hearts, which correlates with the uncoupling of β AR from downstream signalling.²⁵⁶ Mice lacking AC5 exhibit increased longevity, protection against oxidative stress, and protection against cardiomyopathy induced by aging, pressure-overload, or isoproterenol-mediated chronic catecholamine stress.²⁵⁷ On the other hand, overexpression of the AC6 subtype in transgenic mice results in increased cardiac function under basal conditions, without cardiomyopathy. In a cardiomyopathy setting, this overexpression results in increased cardiac function. Additionally, adenoviral gene transfer of AC6 in animals with isoproterenol-induced cardiomyopathy showed improved cardiac output. Finally, transgenic overexpression of AC6 ameliorated cardiac remodelling after myocardial infarction and ischemia-induced HF. Unlike other subtypes, increase in AC6 seems to offer

cardioprotection, rather than having detrimental effects, which could be attributed to the involvement of this subtype in different downstream signalling pathways.^{256,258}

Cell-Based gene therapy

The palliative outcome of current therapeutic strategies, as well as the necessity to address the loss of cardiac cells due to myocardial injury, has inadvertently led to the pursue of cell-based therapies. Cell cardiac therapies aim at rescuing the damaged myocardium or repopulating the heart with new cells. To this end, several cell types have been tested with varying degrees of success. Myocardial regeneration strategies can be divided into 4 major categories: (1) targeting cardiomyocyte division, (2) regeneration by cardiac stem cells, (3) increasing the homing of endogenous stem cells, and (4) regeneration by exogenous, noncardiac stem cells.

1. *Targeting cardiomyocyte division:* Adult cardiac myocytes are terminally differentiated cells with limited potential for regeneration or mitotic division.²⁵⁹ In order for this to happen, several key cellular processes have to be completed: DNA synthesis, genome duplication, karyokinesis, and cytokinesis.²⁶⁰ Cyclins are cyclically expressed proteins that act at restriction checkpoints of cell division.^{259,261} Among the cyclins, cyclin D2 and cyclin A2 have been shown to increase DNA synthesis, mitotic markers, and ultimately survival in HF animal models.^{262–267} However, application of these target genes in a clinical gene therapy setting will be greatly hindered due to safety concerns, as cyclins have long been implicated in the development of cancer²⁶⁸ and their effect can be exerted to other cell types with detrimental consequences.²⁶⁷
2. *Regeneration by cardiac progenitor cells (CPCs):* A review of the literature reveals that endogenous CPCs have been isolated based on the presence of at least 1 of 4 main characteristics: the presence of *c-kit*, *sca-1*, or *islet-1* or the ability to extrude the DNA-binding dye Hoechst 33342.²⁶⁰ All these cell populations have exhibited myogenic or vascularization potential in animal models. CPCs have also been identified in human hearts, although it has been argued that the occurrence is too low to have significant therapeutic prospective.^{260,269}
3. *Noncardiac progenitor cells:* These include epicardial/pericardial stem cells, neural crest– derived cells, perivascular cells, and hematopoietic and mesenchymal stromal stem cells (MSCs). All these cell types have exhibited different degrees of cardiomyocyte lineage potential.²⁶⁰ Of these, MSCs have been shown to offer cardioprotection in different settings, although this effect does not seem to arise from new cardiomyocyte formation.²⁷⁰ Of increasing interest are the paracrine effects of MSCs, through the secretion of growth factors, interleukins, angiogenic factors, and others.²⁷¹ Further studies have focused on modifying MSCs to increase survival via lentiviral overexpression of growth or antiapoptotic factors, such as AKT,^{272,273} Survivin,²⁷⁴ and Bcl-2.²⁷⁵ To enhance their paracrine effect, MSCs have also been genetically engineered with chemokines, such as SDF-1 or its receptor (CXCR4), to enhance the

homing of endogenous stem cells,^{276,277} or with angiogenic factors, such as VEGF, to augment angiogenesis.^{278–280}

4. *Increasing the homing of endogenous stem cells:* Another approach gaining ground in the past years has been the gene transfer of factors that increase the migration and homing of endogenous stem cell populations. After myocardial injury, such as seen in ischemic myocardium, chemokines are secreted to the effect of progenitor cell attraction to the site of injury. This in turn results in increased myocardial repair. The SDF-1–CXCR4 (its receptor) axis has a procardiogenic effect on pluripotent stem cells.^{281,282} Adenoviral expression of SDF-1 in infarcted myocardium offers cardioprotection^{280,283} and clinical trials of naked plasmid gene delivery of SDF-1 to the periinfarct area are currently under way.²⁸⁴ Stem cell mobilization can also be induced by granulocyte-colony stimulating factor (G-CSF), although the success of this factor has been ambiguous.²⁸⁵ Finally, gene delivery of stem-cell factor (SCF) to the infarcted rat heart resulted in increased homing of progenitor cells and ultimately cardiac function.²⁸⁶
5. *Induced Pluripotent Stem Cells (iPS cells):* iPS cells originate from somatic somatic cells following induced expression of specific “reprogramming”-stemness factors (OCT3/4, SOX2, KLF4, and c-MYC) that result in the regression to a more “immature” pluripotent phenotype.^{287,288} The autologous nature of the iPS that can derive from a variety of tissues^{289–292} from the individual, where, after reprogramming, they will be injected, alludes that an immune response is abrogated.²⁸⁷ iPS have been shown to assist in the recovery of cardiac function after infarction.²⁹² Although iPS-derived cardiomyocyte generation has been achieved, their long-term survival in vivo, however, remains elusive. Additionally, the generation of teratoma poses a great safety concern before translating this technology to the clinic.²⁹³

Clinical Trials

Currently, most clinical gene therapy trials target the revascularization of the ischemic myocardium, as chronic HF is most commonly caused by myocardial infarction. These clinical trials are discussed in extend in the angionenic gene therapy section. However, HF can also be attributed to a variety of causes that cannot be managed by angiogenesis. Taking into account the underlying molecular mechanisms of HF, several new gene candidates, which have been identified in extensive preclinical studies, are currently being assessed in ongoing clinical trials.

It is important that the measurements of the efficacy of gene therapy be strong enough to detect biological signals. In pre-clinical animal studies, scientists use invasive hemodynamic measurements along with various imaging modalities to evaluate the physiological effects of various gene therapy strategies. Clinically, the hemodynamic measurements may not be applicable since they are too invasive, especially since they would need to be performed repeatedly. For gene therapy trials where it is expected that a small number of patients would be enrolled, composite clinical end-points need to be used. This includes time to clinical

events such as death, left ventricular assist device, transplant, and HF hospitalizations. In addition, other parameters such as echocardiographic measures, quality-of-life assessments, biomarkers, 6-minute walk tests, and oxygen consumption tests are routinely used in HF trials as secondary end-points.

Based on a large amount of data in preclinical models, a first-in-human, phase 1 trial (CUPID) was recently conducted restoring the levels of this key enzyme in HF patients through gene transfer of the SERCA2a cDNA via a recombinant AAV vector (AAV1.SERCA2a). The results of the phase 1 study demonstrated an acceptable safety profile and improvements at 6 months across symptomatic, functional, biomarker, and left ventricular function/remodelling parameters.⁹² However, even though the phase 1 trial showed biological efficacy, the phase 1 trial was open label and it is hard to interpret these results. The safety profile of phase 1 led to the approval of a double-blind placebo-controlled randomized trial of AAV1.SERCA2a.⁹² The phase 2 trial enrolled 39 patients with advanced HF who were randomized to receive intracoronary AAV1-mediated SERCA2a gene delivery (in 1 of 3 doses—low dose: 6×10^{11} DNase-resistant particles [DRP]; middle dose: 3×10^{12} DRP; high dose: 1×10^{13} DRP) versus placebo and was finally completed. At 6 months, the AAV1.SERCA2a-treated patients demonstrated improvement or stabilization in New York Heart Association class, Minnesota Living With Heart Failure Questionnaire, 6-minute walk tests, VO_2max , N-terminal-pro-brain natriuretic protein levels, and left ventricular end-systolic volumes. Significant increases in time to adjudicated cardiovascular events and a decreased frequency of cardiovascular events per patient were observed for AAV1.SERCA2a. No increases in adverse events, disease-related events, laboratory abnormalities, or arrhythmias were observed in AAV1.SERCA2a-treated patients compared to placebo. Even though larger studies are needed to establish AAV.SERCA2a as a treatment modality for advanced HF, the CUPID trial demonstrated that SERCA2a is a critical target in the pathogenesis of HF.

Two other clinical trials targeting SERCA2a are currently enrolling patients. The first trial is in patients with advanced HF having received a left ventricular assist device at least 1 month prior to treatment and who will receive either AAV6.SERCA2a or saline. This trial is being conducted in the United Kingdom. A second Phase 2 monocentre double-blind randomized placebo-controlled, parallel study will be performed at Pitié-Salpêtrière, Paris, France, with the primary objective to investigate the impact of AAV6-CMV-SERCA2a on cardiac remodelling parameters in patients with severe HF.

In a recent randomized, double-blind phase I/II clinical trial, the safety and efficacy of adenoviral gene transfer of AC6 will be assessed in patients with congestive HF. Currently, patients are being enrolled to receive intracoronary injection of Ad5.hAC6 or sucrose solution (placebo) (ratio 3:1), at 6 different dosages ranging from 3.2×10^9 vp (viral particles) to 3.2×10^{12} vp.²⁹⁴

In another recent nonrandomized phase I clinical trial, ACRX-100 (SDF-1) will be injected directly into the myocardium of patients with ischemic heart disease at 3 different dosages. The naked DNA will be injected as a single dose at multiple sites using a catheter-based percutaneous, left ventricular approach. The aim of the study is to evaluate safety,

tolerability, and preliminary efficacy by documenting all adverse effects at 1 month post injection.²⁸⁴

Conclusion

New molecular-based strategies for restoring calcium cycling may provide new therapeutic strategies for patients with severe HF. The use of gene therapy to target these calcium cycling proteins has proved to be safe and effective for other targets.

Acknowledgments

Funding Sources

This work is supported by Leducq Foundation through the Caerus network (R.J.H.) and National Institutes of Health grants HL-093183, HL-088434, HL-071763, HL-080498, HL-083156, and HL-100396 (R.J.H.).

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Figure 1.

Adeno-associated viral (AAV) endocytosis and intracellular trafficking. The entry of AAV vectors into the cell involves several steps, each of which can affect transduction efficiency. The first step involves the binding to the receptor, co-receptors, or attachment factors (1). Endocytosis, which is primarily receptor mediated, occurs in distinct membrane compartments, identified as clathrin-coated pits, although use of alternative pathways has not been excluded (2). Following endocytosis the AAV vectors are compartmentalized into early endosomes (3), which then mature to late endosomes (4). Acidification of the late endosome is a prerequisite for the release of the AAV (5a). Alternatively, late endosomes fuse with lysosomes resulting in degradation of the vector (5b). The AAVs that escape the endosomal compartment traffic to the nucleus (6), where viral uncoating leads to the single-stranded DNA release (7). The ssDNA is then converted to dsDNA (8) and finally it either forms concatamers (9) or integrated into the host genome (10).⁸⁸

Figure 2.

Calcium signalling during excitation-contraction coupling. AC, adenylyl cyclase; ATP, adenosine triphosphate; β-AR, β-adrenergic receptor; cAMP, cyclic adenosine monophosphate; GRK, G protein-coupled receptor kinase; G_s, stimulatory G protein; I-1, (protein phosphatase) inhibitor-1; K⁺ Channel, voltage-gated potassium channel; LTTC, voltage-operated L-type Ca²⁺ channels of the T tubule; NCX, sarcolemmal sodium/calcium exchanger; Na⁺/K⁺ Exchanger, sodium/potassium exchanger; PKA, protein kinase A; PLN, phospholamban; PP1, protein phosphatase 1; RyR, ryanodine receptor; SERCA, sarcoplasmic/endoplasmic reticulum calcium ATPase; S100A1, S100 calcium-binding protein A1; SR, sarcoplasmic reticulum; TnI, troponin I.

Table 1

Comparison of different gene therapy vectors

Vectors	Nonviral	Adenoviral	Lentiviral	AAV
Pathogenicity	No	Common cold	HIV-related virus	No
Tropism	No	Liver	CD4 ⁺ cells	Heart for cardiotropic AAVs
Receptor	NA	CAR	CD4	HSPG for AAV2, α 2.3 sialic acid for AAV4 and AAV5, α 2.6 Sialic acid for AAV1 and AAV6, EGFR for AAV6 (co-receptor), laminin receptor for AAV9
Cell type	Dividing/nondividing	Dividing/nondividing	Dividing/nondividing	Dividing/nondividing
Expression in vivo	Low transient	High transient	Moderate-long term	Moderate-long term
Insert size, kb	>50	>30	7–7.5	4.7
Genetic manipulation	Easy	Moderate	Moderate	Difficult
Production	Easy	Easy	Difficult	Moderate
Integration	No	No	Yes	Low frequency
Preexisting immunity	No	Yes	Maybe	Yes
Immune response	Low inflammatory	Strong inflammatory	Low to moderate	Low to moderate