Gene Therapy for Heart Failure
Lisa Tilemann, Kiyotake Ishikawa, Thomas Weber, Roger J. Hajjar

Abstract: Congestive heart failure accounts for half a million deaths per year in the United States. Despite its place among the leading causes of morbidity, pharmacological and mechanistic remedies have only been able to slow the progression of the disease. Today's science has yet to provide a cure, and there are few therapeutic modalities available for patients with advanced heart failure. There is a critical need to explore new therapeutic approaches in heart failure, and gene therapy has emerged as a viable alternative. Recent advances in understanding of the molecular basis of myocardial dysfunction, together with the evolution of increasingly efficient gene transfer technology, have placed heart failure within reach of gene-based therapy. The recent successful and safe completion of a phase 2 trial targeting the sarcoplasmic reticulum calcium ATPase pump (SERCA2a), along with the start of more recent phase 1 trials, opens a new era for gene therapy for the treatment of heart failure. (Circ Res. 2012;110:777-793.)

Key Words: gene therapy ■ heart failure ■ adeno-associated vectors

Ongoing preclinical studies are providing a sound scientific basis for the evaluation of gene therapy strategies. Furthermore, the anatomic compartmentalization of the heart and its accessibility by surgical and percutaneous approaches render the myocardium a highly amenable target system for gene therapy.1

Over the last decade, novel molecular mechanisms associated with heart failure have been discovered creating new targets for therapeutic interventions. A number of these promising targets cannot be manipulated through pharmacological means but are ideal for gene therapy approaches.

In this review, we will highlight new strategies for the treatment of heart failure by gene transfer, focusing on the vectors, targets, and delivery methods along with the recent clinical results from early clinical trials.

Gene Delivery
Gene delivery vehicles fall into one of two categories: nonviral or viral gene delivery vectors. The most attractive features of nonviral gene delivery vectors are their safety profile and minimal immunogenicity. The size of the transgene in viruses is limited by the packaging constraints, whereas with plasmid there are no packaging limitations that restrict the transgene size. However, despite extensive efforts to improve transfection efficiencies by combining “naked” DNA with delivery agents such as cationic lipids or by using plasmid DNA in combination with physical methods to enhance gene transfer, insufficient transfection efficiencies remain the Achilles heel of nonviral gene delivery methods.

Given these limitations of nonviral delivery vehicles, intense research has taken place over the past years aimed at harnessing the inherent capacity of viruses to deliver genes to cells. Today, the virus most widely used for cardiovascular gene transfer is adenovirus with a total of 54 trials, 24 of which are ongoing.2 Other viral vectors that have reached the clinical trial phase are derived from retroviruses, Sendai virus, and adeno-associated virus (AAV).2 Given the nonproliferative nature of cardiomyocytes, lentiviruses could be promising vehicles for cardiovascular gene therapy. In contrast to AAV, lentiviral and retroviral vectors integrate their genomes into the host genome. Whereas pseudorandom vector genome integration raises the risk of oncogenic transformation,3 genome integration is essential for stem cell therapy because stem cell therapy, by its very nature, requires an expansion of the stem cells to be effective. This expansion requires multiple rounds of cell division, during which the vector DNA of nonintegrating vectors will be lost due to “dilution” of the vector genomes. In regard to the potential for oncogenic transformation by γ-retroviral and lentiviral vectors, it should be pointed that whereas both γ-retroviral vectors and lentiviral vectors preferentially integrate their genome into transcriptional units, lentiviral vector genome integration is, in contrast to γ-retroviral genome integration, usually more distant from transcription start sites.4 Together with the development of self-inactivating lentiviral vectors, the resulting improved safety profile likely contributed to the approval of clinical trials using lentiviral vectors.4

As can be seen in Table 1, each of the vector systems has its unique properties and vector specific advantages and
limitations. One of the potential limitations of adenoviral vectors is that they can only trigger short term gene expression, although it can be argued that for certain applications—for example, the expression of factors that stimulate cell division—this might be advantageous. The far bigger problem of adenoviral vectors is that they are highly immunogenic. Although in cardiovascular gene therapy trials no adverse effect as devastating as the tragic death of Jesse Gelsinger in a trial for the treatment of ornithine transcarbamylase deficiency with an adenoviral vector encoding the deficient enzyme has been observed, the inflammation caused by adenoviral vectors is of significant concern and probably one of the main reasons for the declining the use of this vector system in clinical gene transfer for cardiovascular diseases.

AAV Vectors in Cardiovascular Gene Transfer

AAV is a small, nonenveloped virus with a linear, single-stranded DNA that belongs to the family Paroviridae, the subfamily Parovirinae and the genus Dependovirus. As its name indicates, AAV depends on a helper virus such as adenovirus or herpes virus for productive replication. Contributing to its beneficial safety profile, AAV is not known to be associated with any human disease. Furthermore, the only cis-elements required for AAV vectors are the inverted terminal repeats that flank the (recombinant) viral genome. As a consequence of this, transduction with AAV vectors does not result in the expression of any viral genes, which likely contributes to their low immunogenicity when compared with most other viral vectors (see below).

For all viruses, infection/transduction starts with the binding of the virus to its receptor(s) (Figure 1). The receptors for some of the AAV serotypes have been described in the literature (see Reference 6 for a description of the AAV receptors). Receptor binding is then followed by endocytosis, which for AAV2 is via the so-called CLIC/GEEC (Claudin-Independent Carriers/GPI-anchored protein Enriched Endocytic Compartment) pathway, whereas for AAV5 it has been reported that endocytosis can occur both by clathrin-coated vesicles or caveolae.

At least for the prototypical AAV serotype, AAV2, and AAV5 endocytosis is followed by transport to the Golgi (Figure 1). Evidence suggests that once in the cytoplasm

---

**Table 1. Vectors Systems Used in Cardiovascular Gene Transfer**

<table>
<thead>
<tr>
<th>Vector</th>
<th>Genome</th>
<th>Persistence</th>
<th>Packaging Capacity</th>
<th>Dividing</th>
<th>Nondividing</th>
<th>Advantages</th>
<th>Disadvantages</th>
<th>Open Trials*</th>
<th>Total Trials*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonviral</td>
<td>DNA</td>
<td>Variable</td>
<td>n/a</td>
<td>Yes</td>
<td>Yes</td>
<td>Low cost, nonpathogenic, limited immune response</td>
<td>Low transfection efficiency, no cardiotropism</td>
<td>40</td>
<td>84</td>
</tr>
<tr>
<td>Ad</td>
<td>DNA</td>
<td>No</td>
<td>Up to 30 kb</td>
<td>Yes</td>
<td>Yes</td>
<td>High transduction efficiency, fast expression kinetics, efficient cardiomyocyte transduction</td>
<td>Immunogenicity, transient expression, no cardiotropism</td>
<td>24</td>
<td>54</td>
</tr>
<tr>
<td>AAV</td>
<td>DNA</td>
<td>Yes</td>
<td>~5 kb</td>
<td>Yes</td>
<td>Yes</td>
<td>Nonpathogenic, efficient cardiomyocyte transduction, persistent gene expression, low immunogenicity</td>
<td>Limited packaging capacity</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>RV</td>
<td>RNA</td>
<td>Yes</td>
<td>8 kb</td>
<td>Yes</td>
<td>No</td>
<td>High transduction efficiencies, stable transduction</td>
<td>Risk of oncogenic transformation, transduces only dividing cells, no cardiotropism</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>LV</td>
<td>RNA</td>
<td>Yes</td>
<td>8 kb</td>
<td>Yes</td>
<td>Yes</td>
<td>High transduction efficiencies, stable transduction</td>
<td>Risk of oncogenic transformation, potential CTL response, no cardiotropism</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Sendai</td>
<td>RNA</td>
<td>No</td>
<td>15</td>
<td>Yes</td>
<td>Yes</td>
<td>Nonpathogenic in humans, no obvious path for genome integration</td>
<td>Transient expression, no cardiotropism</td>
<td>2</td>
<td>2</td>
</tr>
</tbody>
</table>

NV indicates nonviral; Ad, adenovirus; AAV, adeno-associated virus; RV, retrovirus; LV, lentivirus; CTL, cytotoxic T-lymphocyte.

*As of June 2011.
AAVV is imported into the nucleus as an intact particle, presumably through the nuclear pore (Figure 1). Import of viral particle is then followed by capsid uncoating and the release of the viral genome (Figure 1). For transcription of the transgene, the single-stranded AAV genome must then first be converted into a double strand genome (Figure 1).

**Tropism of AAV Serotypes**

To date, 13 AAV serotypes and more than 100 AAV variants have been described in the literature, although most gene transfer experiments are performed with vectors based on AAV1–9. Together, the AAV serotypes display broad, yet serotype-specific tissue and cell tropism (Table 2), which is likely, at least to a certain extent, a result of their diverse receptors (for a compilation see6). Nonetheless— species differences notwithstanding—overall, AAV1, AAV6, AAV8, and AAV9 show strong cardiac transduction, with AAV9 appearing to be the most cardiotropic, at least in rodents. In light of the observed species differences, it is unfortunate that our knowledge of the cardiotropism of the AAV serotypes in nonhuman primates is very limited, although it has been shown that AAV2 is able to transduce cardiomyocytes after intramyocardial injection10 and in Rhe
dus Macaques AAV6 is superior to AAV8 and AAV9 in transducing cardiac tissue when delivered by percutaneous, transendocardial injection.13 Importantly, the results from the recently completed phase II of the so-called CUPID (Calcium Upregulation by Percutaneous Administration of Gene Therapy in Cardiac Disease) indicate that AAV1 can successfully transduce human cardiac tissue.12

**Transductional Targeting**

Despite the substantial cardiotropism of AAV1, AAV6, and AAV9, all of these serotypes transduce additional tissues, at least to some extent. It is therefore not surprising that substantial efforts have been made to develop AAV variants with further enhanced cardiac tropism.

One general approach to alter AAV tropism, including the isolation of AAV variants with cardiotropism, that recently has gained considerable attention is so-called directed evolution (Figure 2). All studies using this method are based on the initial creation of AAV libraries with diverse AAV capsids. One method uses the insertion of a (semi)-random peptide sequence into the AAV capsid. A variation of this method replaces a short segment of an AAV capsid protein,13 usually located in the major capsid protein VP3, with a (semi)-random peptide sequence.

In a different approach to create AAV libraries composed of viruses with diverse capsids, viruses with chimeric capsid proteins, for example, capsid proteins with stretches of amino acids from different serotypes within the same capsid protein, are generated. The method for the generation of these so-called shuffled capsid libraries is outlined in Figure 2A.

Regardless of how the AAV libraries are generated, in the next step the most cardiotropic variants are then selected by either in vitro or in vivo evolution.14 Whereas in vitro selection might be technically simpler, it fails to select for variants that can efficiently overcome barriers, such as escape from the vasculature, that virions only encounter in an in vivo setting. A typical in vivo selection procedure is shown schematically in Figure 2B. Briefly, the viral library is injected into the tail vein of mice, 24–72 hours later the heart is harvested and the capsid DNA amplified by PCR. After subcloning into a wild-type AAV backbone, a secondary viral library is created and the selection procedure repeated. After 1 or more additional rounds of selection, individual clones are isolated and the capsid genes sequenced. Recombinant viruses encoding reporter genes are then produced and used for a detailed characterization of the new cardiotropic AAV variants. Although the method just described resulted in the successful identification of AAV variants with enhanced cardiac tropism,15 it enriches AAV variants that successfully deliver their DNA to the heart, regardless whether they successfully infect cardiac tissue. To overcome this potential limitation, Kleinschmidt et al, after the harvesting of the heart, kept heart slices in organotypic culture and superinfected these slices with adenovirus. This should allow the replication and enrichment of AAV variants that successfully infected cardiac tissue. AAV variants that, for instance, only delivered their genomes to the cytoplasm of cardiomyocytes, on the other hand, will not be enriched.16

Another successful way to enhance cardiac tropism has been used by Samulski et al.17 This group replaced a hexapeptide sequence in the receptor-binding region of the AAV2 capsid with the corresponding peptides of other AAV serotypes and variants. One of the novel AAV2 variants, AAV2i8, which is a chimera between the AAV2 capsid and the AAV8 hexapeptide, shows significant skeletal and cardiac muscle tropism with significantly reduced transduction efficiencies of the liver. Clearly, these novel AAV variants are promising candidates for cardiac gene transfer. It has to be pointed out, however, that in cases in which transduction of cardiomyocytes was compared with transduction by AAV9, the latter always showed the highest transduction efficiency.

**Transcriptional Targeting**

Ideally, transductional targeting alone is sufficient to drive the expression of the transgene exclusively in the heart. Unfortunately, however, AAV vectors that do not transduce noncardiac tissues to some extent are thus far unavailable. An
Table 2. Tropism of AAV Serotypes in Animal Models of Cardiac Diseases

<table>
<thead>
<tr>
<th>Animal</th>
<th>Tissue</th>
<th>Serotype Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse</td>
<td>Heart</td>
<td>iv: AAV9&gt;AAV6&gt;AAV8&gt;AAV4&gt;AAV7&gt;AAV1&gt;AAV3&gt;AAV2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>iv: AAV8&gt;AAV1&gt;AAV2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>iv: AAV9&gt;AAV8&gt;AAV1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>iv: AAV9&gt;AAV8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>iv: AAV9&gt;AAV6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>iv: AAV1&gt;AAV4&gt;AAV5&gt;AAV6&gt;AAV2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ic: AAV6&gt;AAV9&gt;AAV2&gt;AAV1&gt;AAV4&gt;AAV6&gt;AAV7&gt;AAV3&gt;AAV5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>imc: AAV1&gt;AAV2&gt;AAV3&gt;AAV4&gt;AAV5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ipc: AAV9&gt;AAV7&gt;AAV6&gt;AAV8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ip: AAV8&gt;AAV7&gt;AAV1&gt;AAV6&gt;AAV2&gt;AAV5</td>
</tr>
<tr>
<td>Liver</td>
<td></td>
<td>iv: AAV9&gt;AAV7&gt;AAV6&gt;AAV2&gt;AAV5&gt;AAV1&gt;AAV4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>iv: AAV9&gt;AAV8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>iv: AAV9&gt;AAV6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>iv: AAV1&gt;AAV4&gt;AAV5&gt;AAV6&gt;AAV2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ic: AAV7&gt;AAV9&gt;AAV8&gt;AAV2&gt;AAV6&gt;AAV1&gt;AAV4&gt;AAV3&gt;AAV5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ipc: AAV8&gt;AAV7&gt;AAV9&gt;AAV1&gt;AAV6</td>
</tr>
<tr>
<td>Skeletal muscle</td>
<td></td>
<td>iv: AAV6&gt;AAV7&gt;AAV8&gt;AAV9&gt;AAV3&gt;AAV1&gt;AAV2&gt;AAV4&gt;AAV5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>iv: AAV8&gt;AAV1&gt;AAV2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>iv: AAV9&gt;AAV8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>iv: AAV9&gt;AAV6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>iv: AAV5&gt;AAV6&gt;AAV1&gt;AAV4 (AAV2 no expression)</td>
</tr>
<tr>
<td>Rat</td>
<td>Heart</td>
<td>iv: AAV8&gt;AAV1&gt;AAV6&gt;AAV7&gt;AAV4&gt;AAV5 (AAV2, AAAS no expression)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>imc: AAV9&gt;AAV8&gt;AAV7&gt;AAV1</td>
</tr>
<tr>
<td>Canine</td>
<td>Heart</td>
<td>imc: AAV6&gt;AAV9&gt;AAV8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>AAAV8</td>
</tr>
<tr>
<td>Liver</td>
<td></td>
<td>imc: AAV9&gt;AAV8&gt;AAV6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>cv: AAV8&gt;AAV2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ipv: AAV8&gt;AAV2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>AAAV2</td>
</tr>
<tr>
<td>Skeletal muscle</td>
<td></td>
<td>imc: AAV6&gt;AAV9&gt;AAV8</td>
</tr>
<tr>
<td>Swine</td>
<td>Heart</td>
<td>imc: AAAM1&gt;AAV2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>AAV1</td>
</tr>
</tbody>
</table>

in indicates intravenous; ic, intracardial; imc, intramyocardial; ipc, intrapericardial; ip, intraperitoneal; cv, cephalic vein; ipv, intraportal vein.

alternative and complementary approach to restrict transgene expression to the heart is transcriptional targeting, that is, the use of cardiac specific promoters. An additional advantage of cardiac specific promoters is that, in contrast to the most commonly used promoter, the cytomegalovirus (CMV) promoter, their expression is not expected to be downregulated and hence should result in long-term gene expression.

One promoter that was used in both adenoviral and AAV vectors is the ventricle-specific Myosin Light Chain-2 (MLC-2v) promoter. Although this promoter is more than 10-fold less efficient than the already weak RSV promoter, this inadequacy can be partially over come by the inclusion of 4 copies of the 250-bp enhancer fragment of the MLC-v2 gene. This promoter construct drives expression that is only 3.8-fold lower than expression from the strong CMV promoter. For AAV vectors this comes, however, at the price of reducing the allowable size of the transgene by 1000 bp, which is not insignificant in a vector system that has a packaging capacity of ≈5 kb. The Myosin Heavy Chain Promoter, which has been used extensively in the generation of transgenic mice that express transgenes specifically in cardiomyocytes, is not suitable for AAV vectors because it is 5.5 kb long. Unfortunately, expression from a minimal version of the same promoter is much lower than expression from RSV or CMV promoters. Maybe the most promising promoter for cardiac-specific expression that is suitable for AAV vectors is a 418 bp fragment from the chicken cardiac Troponin T promoter. The strength of this promoter is only...
approximately 2-fold lower when compared with the CMV promoter.\textsuperscript{23}

**Regulation of Transgene Expression**

Both the viral and heart-specific promoters mentioned in the previous section are constitutively active, that is, the expression levels of the transgene cannot be regulated. For certain applications, however, it is important that the transgene is either expressed only under certain physiological conditions or that expression levels or the time-frame of expression can be regulated pharmacologically. The most commonly systems that allow the manipulation of expression levels with small molecules are the so-called Tet-on\textsuperscript{24} and Tet-off\textsuperscript{25} and the rapamycin/FK506 inducible\textsuperscript{26} systems. Both these systems as well as a mifepristone (RU486)-inducible system\textsuperscript{27} have been used in context of AAV vectors.

In addition it can be useful to express the transgene under specific pathological conditions. One system that allows the induction of transgene expression under hypoxic conditions is based on the inclusion of a hypoxia response element into the promoter region. Hypoxia-inducible systems have been based on the oxygen-dependent degradation domain of hypoxia-inducible factor 1\textsuperscript{124,25}.

**Gene Silencing With shRNAs and Artificial MicroRNAs**

For many disorders, including cardiac diseases, it is necessary to overexpress a protein because the endogenous protein is either nonfunctional or expressed at insufficient levels. It is equally possible, however, that a disorder is caused by a mutated protein that has dominant effects or that an endogenous protein is expressed at pathologically high levels. Furthermore, in certain instances, it is therapeutically benefi-
general driven by pol III promoters. After export to the cytoplasm they are converted into siRNAs by Dicer and the guide strand is loaded into the RISC, which then results in the degradation of the target mRNA. shRNA expression cassettes can also be incorporated into viral vectors, including AAV vectors. Owing to their ability to drive long-term expression, AAV vector-based shRNA mediated gene silencing results in a lasting suppression of target gene expression.\(^3\)\(^3\) It became soon clear, however, that AAV vector-based gene silencing with shRNAs is not without potential complications. Mark Kay’s group used the strongly hepatotropic AAV serotype 8 to deliver shRNAs directed against luciferase, human α1-antitrypsin or the hepatitis B virus (HBV) genome.\(^3\)\(^4\) Out of 39 shRNAs tested, 36 showed a dose-dependent toxicity, and 23 shRNAs caused individual mice to die. The large number of diverse shRNAs made off-target effects unlikely and further analysis revealed that the toxicity is probably due to saturation of shared components of the shRNA/miRNA pathway, in particular exportin 5.\(^3\)\(^4\) This is probably unlikely and further analysis revealed that the toxicity is probably due to saturation of shared components of the shRNA/miRNA pathway, in particular exportin 5.\(^3\)\(^4\) Importantly, however, the toxicity was both dependent on viral dose and the precise nature of the shRNA sequence\(^3\)\(^4\) suggesting that a careful selection of the shRNA sequence, promotor strength and vector dose should allow to minimize toxicity, at least for certain applications.

**Immune Responses Against Viral Vector and Transgenes**

**Adenoviral Vectors**

As mentioned earlier, adenoviral vectors are highly immunogenic, which is probably a major reason for their declining use in human cardiovascular gene therapy. This is especially pronounced in first- and second-generation adenoviral vectors that express several viral genes as well the transgene.\(^3\)\(^5\) This immunogenicity is partly alleviated with helper-dependent, so-called gutless, adenoviral vectors because they do not express any viral genes.\(^3\)\(^5\) Nonetheless, even though the adaptive, especially cellular, immune response with gutless adenoviral vectors is attenuated, they still elicit a strong innate immune response. That an innate immune response against adenoviral vectors can have catastrophic consequences was demonstrated in a trial with a second generation adenoviral vector that resulted in the death of a trial participant.\(^3\)\(^5\) An additional complication for the use of adenoviral vectors is the high prevalence of neutralizing antibodies against adenoviruses. Approximately 97% of the population harbor neutralizing antibodies against type C adenoviruses, which include the commonly used Ad2 and Ad5.\(^3\)\(^6\)

Furthermore, although helper-dependent adenoviral vectors show reduced myocardial inflammation after intramyocardial injection when compared with first-generation adenoviral vectors, the adenoviral capsid also appears to modulate the immune response against the transgene.\(^3\)\(^7\) Thus, whereas AAV-mediated GFP expression is long-lasting, both after intramyocardial and intrapericardial injection,\(^3\)\(^7\) expression from both first generation and helper-dependent, that is, gutless, adenoviral vectors was transient.\(^3\)\(^7,\)\(^3\)\(^8\) In the future, it might be possible to devise strategies to overcome immune responses against either the adenoviral capsid or transgene but, at present, these immune reactions clearly pose a serious obstacle to the use of adenoviral vectors in cardiovascular gene therapy.

**Lentiviral Vectors**

Because of their ability to transduce nondividing cells and their potential to trigger long-term transgene expression, lentiviral vectors represent a attractive gene delivery system for cardiovascular gene therapy. In contrast to adenoviral and AAV-based vectors, neutralizing antibodies against lentiviral vectors are usually not of concern. However, depending on the origin of the envelope protein used to create the pseudotyped lentiviral vectors, as well as the route of vector administration, a strong cytotoxic immune response can be generated.\(^3\)\(^6\) In general, this immune response is directed against the transgene, although a potential immune reaction against the envelope proteins cannot be dismissed.\(^3\)\(^6\) The CTL-mediated destruction of transduced cells is probably due to fact that lentiviral vectors efficiently transduce antigen presenting cells such as Kupffer cells, splenic macrophages, and dendritic cells.\(^3\)\(^6\) Although in one report pseudotyping a feline immunodeficiencyp vector with the baculovirus envelope protein gp64 has been shown to overcome this limitation resulting in long-term transgene expression,\(^3\)\(^9\) in another mouse strain, gene transfer with a gp64-pseudotyped HIV-derived vector resulted only in transient (6 months) transgene expression.\(^4\)\(^0\) Whereas the use of tissue-specific promoters can mitigate the expression of the transgene in antigen presenting cells, at least under certain circumstances, these promoters appear not to be sufficiently tissue specific to prevent transgene expression in all antigen presenting cells.\(^3\)\(^6\)

A clever approach to overcome this problem was developed by Naldini et al.\(^4\)\(^1,\)\(^4\)\(^2\) In this system, several copies of an miRNA target sequence that is perfectly complementary to an miRNA that is highly and specifically expressed in hematopoietic cells are included into the 3‘-untranslated region of the transgene mRNA.\(^4\)\(^2\) These miRNA target sequences are recognized by the endogenous miRNA in antigen presenting cells resulting in the degradation of the transgene mRNA in these cells but not in target cells. That this is a promising approach has been shown in a mouse model of factor IX deficiency. In this system, a lentiviral vector triggered long-term factor IX expression.\(^4\)\(^1,\)\(^4\)\(^2\)

**AAV Vectors**

**Pre-existing Neutralizing Antibodies**

Regarding the effect of the immune system on transduction by AAV vectors, the comparatively high incidence of pre-existing neutralizing antibodies in the general population is probably the most concerning. Although the prevalence of neutralizing antibodies varies with serotypes and geographic region, most studies report the presence of neutralizing titers of ≥1:20 in 20% to 40% of the population.\(^4\)\(^3\)–\(^4\)\(^5\) The cutoff of titers ≥1:20, however, is not only arbitrary, it is also likely clinically insufficient as an exclusion criteria. Both studies in humans\(^1\)\(^2,\)\(^4\)\(^6,\)\(^4\)\(^7\) and animals\(^4\)\(^8\)–\(^5\)\(^1\) demonstrated that titers as low as 1:4 or even 1:2 can significantly influence or eliminate transgene expression or affect clinical results. For instance, in a clinical trial aimed at treating factor IX deficiency, in the high-dose group with a liver-directed AAV vector encoding...
factor IX, 1 individual with a neutralizing titer of 1:2 showed factor IX expression, albeit only transiently. A second patient with a titer of 1:17, on the other hand, showed no factor IX expression.\textsuperscript{47} In the CUPID trial, 1 exclusion criterion was neutralizing titers of >1:2.\textsuperscript{12,46} In this trial, many patients in the high-dose group of AAV1.SERCA2a showed clinical improvements. However, 2 individuals in the high-dose group that were originally judged to harbor no neutralizing antibodies later tested positive for neutralizing antibodies, and these patients did not show any improvement.\textsuperscript{12,46} Importantly, because patients with antibody titers as low as 1:4 were excluded from the CUPID trial, nearly half the prospective patients could not be enrolled because their antibody titers were too high.\textsuperscript{12,46} This illustrates that future work is needed to allow the inclusion of most if not all potential patients into clinical trials based on AAV vectors.

**Innate Immune Response**

In contrast to adenoviral vectors, AAV does not induce a strong immune response, although systemic injection can give rise to a transient increase in inflammatory cytokines.\textsuperscript{52} Furthermore, it has been demonstrated that AAV can interact with factors of the complement cascade and that these interactions are essential for the endocytosis of AAV by macrophages.\textsuperscript{53} Nonetheless, although it is clear that the innate immune system can play a role in the host response to AAV infection, this response is not nearly as strong as the innate immune response against adeno viral vectors.

**Cellular Immune Response Against the Transgene and the AAV Capsid**

In gene therapy applications in which a missing protein is replaced, a significant risk for an immune response against the transgene exists. Fortunately, however, in cardiovascular gene transfer the transgenes to be expressed are almost exclusively proteins that are also expressed under physiological conditions but the level of expression is upregulated or downregulated during disease progression. Because of this, the host developed tolerance against these proteins and an immune response against the transgene is highly unlikely.

A priori, a cellular immune response against the AAV capsid would seem unlikely because AAV vectors do not express any viral genes. However, it became clear, in an otherwise successful clinical trial to treat hemophilia B, that cellular immune responses against the AAV capsid can occur and that such cellular immune responses can result in the destruction of transduced cells.\textsuperscript{47,54} More recently, however, it was demonstrated that such an immune response can be successfully suppressed by a short course of prednisolone.\textsuperscript{55} Furthermore, for cardiovascular gene therapy it is important to point out that in the only cardiovascular gene therapy trial using AAV vectors, the CUPID trial, such a cytolytic T-cell response was not observed.\textsuperscript{12,46} In this trial, only one patient showed a transient positive signal in an ELISPOT assay indicating a cellular immune response against the AAV1 capsid and this was concurrent with an influenza infection.\textsuperscript{46} Nonetheless, it seems advisable to monitor such responses carefully in cardiovascular or other gene therapy trials with AAV vectors.

**Gene Delivery**

**Antegrade Arterial Infusion**

Percutaneous coronary artery catheterization is a minimally invasive and well-established procedure that allows homogenous gene delivery to each territory of the heart. The major advantages of this approach are that it is minimally invasive and relatively safe. Thus, it is especially attractive for patients with end-stage heart failure. However, gene delivery can be impeded in patients with severe coronary artery disease. More importantly, there is some variability regarding the efficiency of antegrade coronary gene transfer, partly related to the relatively fast transit of the vector through the vasculature. In a large animal model of volume-overload–induced heart failure, antegrade coronary gene transfer (Figure 3A) was shown to significantly restore cardiac function. Infusion through the lumen of an inflated angioplasty catheter with temporary occlusion of the coronary artery (Figure 3B) may increase myocardial gene expression but remains controversial. In a study by Boekstegers et al, ischemia during coronary artery infusion did not significantly increase myocardial transduction.\textsuperscript{56} In contrast, infusion with temporary coronary occlusion at a considerably higher flow rate and consequently elevated coronary pressures resulted in higher gene expression, but was also associated with more myocardial injury.\textsuperscript{57}
An approach that focuses on enhancing the vector residence time in the coronary circulation is coronary venous blockade. Antegrade coronary infusion with a short occlusion of both a coronary artery and a coronary vein enhanced myocardial gene expression\(^{56,59}\) (Figure 3C). This method preserved left ventricular function and inhibited ventricular remodeling in a large animal model of heart failure.\(^{59}\) However, even a short ischemia period should be considered as carrying a risk in patients with advanced heart failure.

To maximize the duration of vector exposure to the endothelium and at the same time minimize systemic distribution, Kaye and colleagues developed an extracorporeal device that drains blood from the coronary sinus using an occlusion catheter and returns the oxygenated coronary venous blood to the left main coronary artery via a peristaltic pump (V-Focus, Osprey Medical Inc, St Paul, MN).\(^{60,61}\) In an ovine model of tachycardia induced heart failure, the closed loop recirculation method was more efficient in the transduction of cardiomyocytes than antegrade coronary infusion, which also translated into a greater improvement of left ventricular function.\(^{60,61}\) Most of the approaches to improve the efficiency of coronary artery infusion are based on increasing the exposure time of the vector to the endothelium. However, transduction efficacy correlates with coronary flow as well as exposure time and vector concentration.\(^{62}\) Based on the former, antegrade coronary artery infusion supported by means of an increased coronary flow, for example, an intra-aortic balloon pump might further enhance cardiac gene transfer.\(^{63}\)

Antegrade coronary artery infusion might be the safest percutaneous gene delivery method, but at the cost of efficiency relative to other techniques. However, to date, all optimization strategies implicate potential damage to the myocardium, limiting their clinical application.

### Retrograde Venous Infusion

The coronary venous system provides an alternate route for the percutaneous delivery of therapeutic agents to the myocardium. In a clinical setting, this approach is attractive for patients with impaired coronary artery circulation and limited potential for revascularization. Access to the myocardium can be achieved with this approach regardless of atherosclerosis severity or coronary artery obstruction.

Numerous studies showed that retrograde coronary venous infusion is an alternate myocardial delivery method for cardioprotective drugs.\(^{64,65}\) Shortly thereafter, retrograde venous infusion was also explored as a method for gene transfer. The rationale for a high transduction efficiency is based on controlling the exposure time of vector to the endothelium and on increasing the pressure gradient of capillary filtration.\(^{56,66}\)

Indeed, studies in large animal models demonstrated that an efficient and homogenous myocardial transduction can be achieved by retroinfusion into the coronary venous system.\(^{56,67,68}\) Boeksteger et al showed that gene expression after pressure-regulated retrograde venous infusion was significantly higher than after antegrade coronary delivery if the retroinfusion was accompanied by simultaneous induced ischemia (Figure 4B). Compared with percutaneous or surgical direct myocardial injection, retrograde venous infusion also achieved a more homogeneous and efficient reporter gene expression.

Similar to antegrade coronary artery infusion, a closed loop recirculation retrograde venous infusion approach is also feasible. Recently, the Bridges group demonstrated an extremely high transduction efficiency in the majority of cardiac cardiomyocytes in sheep while minimizing collateral organ exposure, using a retrograde recirculation method during cardiopulmonary bypass surgery.\(^{69}\)

Retrograde coronary infusion has been shown to be safe when performed by a trained and experienced surgeon. Trauma to the coronary veins, a potential complication, could be minimized with modifications and improvements in catheter design. However, the procedure is not completely without risks. Highly elevated coronary venous pressure can result in myocardial edema or hemorrhage. Careful pressure monitoring should always be used to avoid this complication. Though with the growing demand for cardiac resynchronization therapy, cannulation of the coronary veins has become a routine procedure, monitoring of the pressure regulation adds to the complexity of this procedure. More importantly, the ischemia during the infusion might not be well tolerated in patients with advanced heart failure.

In summary, percutaneous retrograde coronary infusion is an effective, minimally invasive procedure but a relatively complex approach to gene delivery specifically to the myocardium. This approach requires some expertise and meticulous pressure monitoring to be performed safely.

### Aortic Cross-Clamping

In rodents, the vector is injected into the aortic root whereas the aorta and the pulmonary artery are cross-clamped for a few heart beats.\(^{70}\) Whereas cross-clamping the beating heart is only relatively simple and routine in rodents, a modified...
version of this technique has been developed for large animals. In comparison to rodent models, large animals studies using aortic cross-clamping are complex surgical procedures that require cardiac arrest and a complete surgical isolation of the heart in situ with cardiopulmonary bypass.\textsuperscript{71,72}

Virus injection into the aortic root during cardiopulmonary bypass (CBP) with a cross-clamped aorta resulted in selective myocardial uptake and expression in piglets.\textsuperscript{71} To further increase the efficacy of this approach, Bridges and colleagues developed and refined a CBP platform with selective retrograde coronary sinus infusion and recirculation of the vector through the heart. This technique yielded extensive transgene expression in a significant percentage of cardiac myocytes.\textsuperscript{72,73}

Notwithstanding the very high efficiency of the advanced aortic cross-clamping techniques in large animals, their highly invasive nature and the associated potential morbidity limits a possible translation into clinical studies to patients undergoing cardiac surgery for other reasons.

**Intravenous Infusion**

Intravenous injection is the simplest and least invasive method among current available methods of cardiac gene delivery. In rodents, injection into the tail vein results in successful cardiac gene expression. However, as of today, application of this method is feasible only in small animals, mostly due to limited cardiac specificity of presently available vectors. Although dilution by the systemic blood circulation compromises the vector concentration in the cardiac circulation, uptake by other organs such as liver, lung, and spleen before the vectors reach the heart is another issue. Bypassing the liver and the spleen by injecting the vectors into the pulmonary artery did not improve the efficacy. Development of highly cardiotropic vectors without any harmful effects on nontargeted organs, together with methods to augment cardiac uptake, such as ultrasound targeted microbubble destruction,\textsuperscript{74,75} may expand the application of intravenous injections to larger animals and eventually to humans.

**Direct Intramyocardial Injection**

Intramyocardial injection is one of the most widely used gene transfer methods, ranging from small animal studies to clinical trials focusing on cardiac angiogenesis. The vectors are injected either endocardially (Figure 5A) or epicardially (Figure 5B) into the target area with a small gauge needle. The primary advantage of this method is that vector delivery bypasses the endothelial barrier. This results in a high local concentration at the injection site. In addition, by avoiding exposure to the blood, deactivation of the vectors by circulating DNAses or neutralizing antibodies can be prevented. Furthermore, there is minimal exposure of the vector to off target organs, although local administration cannot completely avoid some systemic vector distribution.\textsuperscript{76,77}

The simplest approach, however invasive, is the injection during the thoracotomy. Surgical delivery offers direct visual confirmation, which allows precise control of the injection site, including an infarct border.

Another method of intramyocardial injection is based on a composite catheter system (TransAccess).\textsuperscript{78} This delivery technique utilizes an intravascular ultrasound device for catheter guidance to the injection site before the vector is injected transvenously with an extendable needle. However, coronary sinus accessibility limits the cardiac regions affected by this approach.

The endocardial approach requires a catheter with a retractable injection needle and imaging guidance modality for determining the injection site. This includes electric mapping systems,\textsuperscript{79} fluoroscopy,\textsuperscript{79} echocardiography,\textsuperscript{79} and MRI.\textsuperscript{80} Currently, the NOGA electromechanical mapping system is the most commonly used guiding system that is also used in clinical angiogenesis trials.

Intramyocardial injection is attractive for local gene delivery. However, the application of this method for heart failure, in which cardiomyocytes are globally impaired, might be limited by a circumscribed target area and inhomogeneous expression profiles.\textsuperscript{67,81}

**Pericardial Injection**

Intrapericardial delivery is performed surgically in rodents,\textsuperscript{82} whereas for larger animals, a percutaneous approach is available as well (Figure 5C). The percutaneous pericardial puncture has been proven to be feasible and safe when guided by imaging techniques such as fluoroscopy and intravascular ultrasound. The percutaneous access to the pericardial space can be achieved minimally invasive via a substernal/xiphoidal puncture.
The pericardial space faces most of the cardiac wall except the septum. In heart failure, in which widespread cardiac gene transfer with little systemic distribution is desired, this large-scale interface combined with the concept of a closed compartment can be a major advantage. These features potentially enable prolonged vector persistence, a slow release over time, a high vector concentration, and minimal leakage to nontarget organs. However, tightly joined pericardial cells restrict transfection to superficial myocardial layers.82–84 This limitation can be partly overcome by the coadministration of various pharmacological agents. Proteolytic enzymes and polyethyleneimine have been shown to increase the penetration depth of the vectors and to allow progressive release, often at the cost of cardiac toxicity.82,85 Although vectors are injected into a closed space, some studies reported extracardiac gene expression, probably due to the rapid turnover of the pericardium fluid through the lymphatic absorption.

Other Delivery Methods
Most cardiac gene transfer studies focus on the left ventricle. In contrast, a recently developed surgical gene “painting” method achieved transmural gene transfer in both atria without affecting ventricular cardiomyocytes.86 Although potentially interesting for targeting common atrial diseases such as atrial fibrillation, this approach might be difficult to translate into a less invasive delivery method.

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. We will discuss in this part some of the most important systems targeted to restore the function of failing cardiomyocytes. For targets to be validated, it is important that they rescue function in animal models when heart failure has been already established, that the rescue is not associated with arrhythmogenesis and that a gene-dose effect is established, for example, with increasing expression of the gene of interest, there is a concomitant improvement in function. Excitation-contraction coupling is dysregulated at multiple levels in the development of heart failure. For this reason, the various channels, transporters, and critical proteins have been targeted pharmacologically and by genetic editing to restore contractile function. In Figure 5, the various targets in excitation-contraction coupling are presented.

Targeting the β-Adrenergic System
The β-adrenergic signaling is adversely affected by multiple changes which lead to β-adrenergic receptor (β-AR) downregulation and desensitization. Upregulation of the critical G-protein–coupled receptor kinase (GRK2) appears to precipitate the abnormalities in β-AR signaling abnormalities. 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 had severe cardiomyopathy.87 In contrast, mice with cardiac overexpression of β2-AR demonstrated increased basal myocardial adenyl cyclase activity with increased left ventricular function.88 Both direct and intracoronary myocardial delivery of adenovirus containing the human β2-AR transgene has resulted in enhanced cardiac performance in rodents and other mammalian models.89,90

Inhibition of 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 binds to the Gbg subunit of activated G proteins phosphorylating β-ARs, which then attach to an inhibitory protein β-arrestin. GRK2 is the most highly 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.91 Studies in mice, in which heart failure (HF) was induced by a myocardial infarction, showed that selective GRK2 ablation 10 days after infarction resulted in increased survival, halted ventricular remodeling, and enhanced cardiac contractile performance.92 A peptide termed βARKct capable of inhibiting GRK2-mediated β-AR desensitization has been evaluated in vivo in animals. Using intracoronary adenovirus-mediated βARKct transgene delivery to rabbits 3 weeks after induced myocardial infarction demonstrated a marked reversal of ventricular dysfunction.57 More recent studies have focused on overexpressing βARKct in large animal models.93

Activation of Cardiac Adenyl-Cyclase Expression
Although detrimental outcomes were demonstrated with multiple elements of the β-adrenergic system used to increase the levels of cyclic adenosine monophosphate (cAMP), activation of adenylyl-cyclase (AC) type VI (AC VI) appears to have a uniquely 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.94 In a pacing model of HF in pigs, intracoronary delivery of adenovirus encoding AC VI resulted in improved left ventricular (LV) function and remodeling, associated with increased cAMP generating capacity.95 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.96

Targeting Ca2+ Cycling Proteins
HF is characterized by multiple defects in Ca2-handling proteins involved in excitation-contraction coupling (Figure 6). 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
More than 20 years ago, Gwathmey et al first reported that calcium cycling is abnormal in human heart failure,97 and it
was found to be partially due to decreased SERCA2a activity regardless of the etiology of the heart failure. Improvement in cardiac contractility after gene transfer of SERCA2a has been demonstrated in a large number of experimental models of heart failure. 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. Beyond their effects on enhancing contractility, SERCA2a gene transfer has been shown to restore the energetics state of the heart, both in terms of energy supply and utilization, decrease ventricular arrhythmias, and enhance coronary flow through activation of eNOS in endothelial cells.

**Phospholamban Inhibition**

Another approach to improve Ca\(^{2+}\)/H\(^{+}\) handling involves inhibition of phospholamban (PLN). Decreasing PLN in human cardiac myocytes showed an improvement in contraction and relaxation velocities similar to the benefit seen with gene transfer of SERCA2a. An AAV9-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. Silencing of PLN expression in a sheep HF model resulted in improved SERCA activity along with improved systolic and diastolic LV function. 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.

**Active Protein Phosphatase Inhibitor-1 and Inhibition of PP1**

HF is associated with elevated PP1 activity in humans resulting in dephosphorylation of PLN. Overexpression of PP1 or ablation of protein phosphatase inhibitor-1 (I-1) in murine hearts has been associated with decreased β-AR–mediated contractile responses, depressed cardiac function, and premature death consistent with HF. Expression of a constitutively active I-1 in transgenic mice led to PP1 inhibition with increased phosphorylation of PLN and improved cardiac contractility. A recent study in transgenic mice expressing constitutively 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.

**S100A1**

S100A1 is part of a family of Ca\(^{2+}\)/H\(^{+}\)-modulating proteins implicated in intracellular regulatory activities. S100A1 is the most abundant S100 protein isoform in the heart. It promotes cardiac contractile and relaxation function through enhancing the activity of both RYRs and SERCA2a. In a rat model of HF, AAV6-mediated long-term expression of S100A1 resulted in a sustained in vivo reversal of LV dysfunction and remodeling. More recently, AAV9 gene transfer of S100A1 in a preclinical model of ischemic cardiomyopathy induced dramatic improvements in contractile function, reinforcing the rationale that a clinical trial of S100A1 gene therapy for human heart failure should be forthcoming.
Small Ubiquitin-Like Modifier Type 1

Recently, Kho et al reported that the levels and activity of SERCA2a in cardiomyocytes are modulated in parallel with the levels of a cytoplasmic protein, small ubiquitin-like modifier type 1 (SUMO1). SUMOs are a family of peptides that alter the function of other proteins in cells through a post-translational modification described as sumoylation. Sumoylation is involved in the modulation of various intracellular processes.

Kho et al found that sumoylation enhanced the stability of SERCA2a in the cell as well as increased its activity. SUMO1 levels were reduced in murine and pig models of heart failure and in failing human ventricles. Increasing SUMO1 levels by AAV9 gene transfer led to a restoration of SERCA2a levels, improved hemodynamic performance, and reduced mortality among the animals with heart failure.

Homing of Stem Cells

The SDF1/CXCR4 complex has emerged as a therapeutic target in ischemic heart failure due to the ability of the SDF-1-CXCR4 system to promote the homing of stem cells to infarcted myocardium. A clinical trial is underway to investigate the therapeutic benefit of SDF-1 overexpression in ischemic cardiomyopathy. In parallel, existing literature highlights the direct effects of CXCR4 on the myocardium and the cardiac myocyte. SDF-1 was shown to decrease myocardial contractility ex vivo and on cardiac myocytes. One recent report has shown increased ischemia-reperfusion injury in rat hearts overexpressing CXCR4, whereas another report investigated the modulation of β-adrenergic receptor signaling by SDF-1 and CXCR4, raising interrogations over the potential complex interaction between these chemokines and the cardiovascular system. Pim-1 kinase has also been shown to enhance survival, proliferation, trafficking, lineage commitment, and functional engraftment of cardiac progenitor cells. Pim-1 is unique as it mediates not only proliferation but also lineage commitment and functional engraftment in hearts.

Targeting Cell Death

Apoptosis is a process of programmed cell death that is involved in normal organ development. In models of acute and subacute ischemia/reperfusion, overexpression of the antiapoptotic protein Bcl-2, Akt, or PI3 kinase reduces the rate of cardiomyocyte apoptosis and improves heart function. In ischemia/reperfusion injury, in which apoptosis plays an important part of myocardial damage, gene therapy with prosurvival factors appears to be amenable to intervention. For these situations, sophisticated promoters with oxygen sensing and modified hypoxia-inducible factor 1α promoters have been designed to induce survival factors in the setting of ischemia. It is less clear if other forms of cardiac injury, such as hypertrophy and HF, can benefit from anti-apoptotic strategies.

Clinical Trials

Despite early failures, gene therapy trials for various diseases, most notably inherited blindness (whereby gene transfer by AAV vectors partially restored vision in a pediatric patient with Leber congenital amaurosis, a major cause of congenital blindness), cancer, infectious diseases, monogenic diseases, and cardiovascular diseases are underway.

In heart failure, there are currently a number of trials ongoing or in the planning stages targeting various pathways for rescuing the failing myocardium. The targets that have been taken forward toward clinical trials include SERCA2a, adenylyl cyclase type 6, and SDF-1.

The first clinical trial of gene therapy in patients with HF was launched in the United States in 2007. CUPID was 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. Twelve-month follow-up of these patients showed an acceptable safety profile. Improvement was detected in several patients, reflected by symptomatic (5 patients), functional (4 patients), biomarker (2 patients), and LV function/remodeling (6 patients) parameters. In the phase 2 trial, 39 patients with advanced HF were randomly assigned to receive intracoronary adeno-associated virus 1 (AAV1)-mediated SERCA2a gene delivery (in 1 of 3 doses (low dose: 6×10^{11} DRP; middle dose: 3×10^{12} DRP; and high dose: 1×10^{13} DRP) versus placebo. Patient symptoms (New York Heart Association class, Minnesota Living With Heart Failure Questionnaire [MLWHFQ]), functional status (6-minute walk test [6MWT] and VO_{2} max), NT-proBNP levels, and echocardiographic measures were evaluated over 6 months. Treatment success was determined by examining concordant trends in the above end points for group- and patient-based comparisons, as well as clinical outcomes. The AAV1.SERCA2a high-dose group met the prespecified criteria for success at the group and individual patient levels. AAV1.SERCA2a-treated patients, versus placebo, demonstrated improvement or stabilization in New York Heart Association class, MLWHFQ, 6MWT, VO_{2} max, NT-proBNP levels, and LV end-systolic volumes. Significant increases in time to adjudicated cardiovascular events, and a decreased frequency of cardiovascular events per patient was observed in all patients receiving AAV1.SERCA2a. No increases in adverse events, disease-related events, or laboratory abnormalities were observed in AAV1.SERCA2a-treated patients compared with placebo. Arrhythmias have been a concern with the overexpression of SERCA2a; however, in the CUPID trials, there were no increases in arrhythmias as measured by implantable cardioverter-defibrillator. Two other clinical trials targeting SERCA2a are currently enrolling patients. The first trial is in patients with advanced heart failure having received left ventricular assist devices at least 1 month before treatment and who will receive either AAV1.SERCA2a or saline. This trial is being conducted in the United Kingdom. A second Phase 2, monocenter, double-blind, randomized, placebo-controlled, parallel study will be held in the Institut de Cardiologie Pitit-Salpêtrière, Paris, France, with the primary objective to investigate the impact of AAV1.SERCA2a on cardiac remodeling parameters in patients with severe heart failure.

In a separate clinical study, adenovirus-5 encoding human adenylyl cyclase type 6 is being delivered through intracoronary injection to patients with congestive heart failure. The
patients will be randomly assigned in a dose-dependent fashion starting at $3.2 \times 10^7$ viral particles to $3.2 \times 10^{12}$ viral particles in 6 dose groups, using a 3:1 randomization fashion with PBS (buffered saline being used for control). The trial is currently enrolling patients.

An additional trial is examining the effects of injecting SDF-1 directly into the myocardium of patients with ischemic heart disease. An open-label dose escalation study to evaluate the safety of a single escalating dose of SDF-1 administered by endomyocardial injection to cohorts of adults with ischemic heart failure is currently enrolling patients. SDF-1 naked DNA will be injected directly into the myocardium at multiple sites through a percutaneous left ventricular approach.

Conclusions

With a better understanding of the molecular mechanisms associated with heart failure and improved vectors with cardiotropic properties, gene therapy can now be considered as a viable adjunctive treatment to mechanical and pharmacological therapies for heart failure. In the coming years, more targets will emerge that are amenable to genetic manipulations, along with more advanced vector systems, which will undoubtedly lead to safer and more effective clinical trials in gene therapy for heart failure.

Sources of Funding

This work was supported by grants NIH R01 HL093183, HL088434, and F20HL100396.

Disclosures

R.H. is a scientific founder of Celladon Co, which plans to commercialize AAV1.SERCA2a for the treatment of heart failure.

References


Gene Therapy for Heart Failure
Lisa Tilemann, Kiyotake Ishikawa, Thomas Weber and Roger J. Hajjar

doi: 10.1161/CIRCRESAHA.111.252981

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2012 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/110/5/777

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation Research can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation Research is online at:
http://circres.ahajournals.org/subscriptions/