Published in final edited form as:
J Mol Cell Cardiol. 2011 May ; 50(5): 759–765. doi:10.1016/j.yjmcc.2010.07.022.

Gene Therapy Strategies for Cardiac Electrical Dysfunction

Ian Greener, PhD and **J. Kevin Donahue, MD**
Heart and Vascular Research Center, MetroHealth Campus, Case Western Reserve University School of Medicine

Abstract

Cardiac disease is frequently associated with abnormalities in electrical function that can severely impair cardiac performance with potentially fatal consequences. The available therapeutic options have some efficacy but are far from perfect. The curative potential of gene therapy makes it an attractive approach for the treatment of cardiac arrhythmias. To date, gene therapy research strategies have targeted three major classes of cardiac arrhythmias: 1) ventricular arrhythmias, 2) atrial fibrillation, and 3) bradyarrhythmias. Various vehicles for gene transfer have been employed with adeno-associated viral gene delivery being the preferred choice for long-term gene expression, and adenoviral gene delivery for short-term proof of concept work. In combination with the development of novel delivery methods, gene therapy may prove to be an effective strategy to eliminate the most debilitating of arrhythmias.

Introduction

The heart's unique electrical properties drive the coordinated and dynamic nature of the heart beat. This requires exquisite coordination of a variety of ionic currents, gap junctions and calcium handling proteins, among others, to maintain an organized electrical rhythm at rest and during exercise. Cardiac disease is associated with abnormal electrical rhythms or arrhythmias, which severely impair cardiac performance and can often be fatal. The global significance of cardiac arrhythmias cannot be understated. In the developed world, sudden cardiac arrest is the leading cause of death. Furthermore, the prevalence of atrial fibrillation and its association with aging highlights it as a disease of major clinical importance. Unfortunately, after decades of effort and vast expenditure, traditional pharmacological approaches have failed to alleviate this burden and can even worsen the situation by inducing rather than preventing arrhythmias. Clearly, there is a considerable need for novel and more efficacious therapies.

In the early 1990's gene therapy was met with great expectation given its curative potential and possibility of specific, localized action that should significantly reduce side-effects. The ‘human genome project’ provided the sequence of every gene in the human body, thereby supporting strategies aiming to use genetic manipulation. However, progress in the field has been somewhat slow with some major setbacks since its inception. The most notable being

Address for Correspondence: J. Kevin Donahue, MD, Heart and Vascular Research Center, MetroHealth Hospital, Case Western Reserve University School of Medicine, Rammelkamp 653, 2500 MetroHealth Drive, Cleveland, OH 44109, TEL (216) 778-7342, FAX (216) 778-1261, kdonahue@metrohealth.org.

Disclosures: Dr. Greener has no disclosures. Dr. Donahue is a founder and director of Excigen, a gene therapy company.

the death of Jesse Gelsinger due to a severe immune response to therapy during a small scale clinical trial in 1999.

As with any novel therapy, improvements continue to be made and problems overcome. Gene therapy is no exception: less immunogenic viral vectors are already in clinical trials, innovative delivery methods are being tested, and we are continuing to further our understanding of the most complex arrhythmia mechanisms down to the genetic level. Taken together, gene therapy is progressing well toward its goal as a viable treatment option in the clinic for the most debilitating of cardiac arrhythmias. This review provides a succinct analysis of currently available methods for gene therapy, their utilization for successful modification of cardiac electrophysiology including choice of target genes, and an assessment of the translational potential of the approach.

General Principles of Myocardial Gene Transfer

Basic elements common to all gene therapy approaches include selection of a gene transfer vector and a delivery method. Other considerations, including the therapeutic gene, target, and genetic control elements, are less generalizable and must be individualized to the specific application.

Vectors

Vectors are vehicles for transport of the genetic material (transgene) into the target cells. Gene delivery vectors can be divided into viral and nonviral types. Nonviral vectors are DNA plasmids with or without complexing agents to increase probability of cellular uptake (calcium phosphate, liposomes, proteins, etc.). The initial gene transfer studies used naked plasmid DNA to show proof-of-concept that genes could be taken up and expressed by tissues, but these early studies also demonstrated the inefficiency of DNA vectors; only a negligible percentage of cells expressed reporter genes after DNA transfection.

The increased efficiency of viral vectors allowed them to quickly supplant DNA as the gene transfer vehicles of choice. Viral vectors are essentially wild type viruses with genetic modifications to prevent viral reproduction or pathology and to insert the transgene. Adenovirus serotype 5 (Ad) and adeno-associated viruses (AAV) have been the most widely used and most successful vectors for myocardial applications. Both vectors can efficiently transduce cardiac myocytes.

Adenovirus is a double-stranded DNA virus with a 74-nm icosahedral protein coat containing a genome of approximately 36 kb. AAV are a phylogenetically distinct class of viruses that are related to adenoviruses in name only. AAV have a 20-nm diameter protein coat and a 5-kb single-stranded DNA genome consisting of only 2 genes: *rep* and *cap*. Advantages of adenoviruses include ease of production and amplification; disadvantages include a limited duration of gene expression (3 to 4 weeks in vivo) and toxicity from the immune response to the virus. The chief advantage of AAV vectors is the possibility of long-term (potentially permanent) gene expression. Disadvantages include cumbersome production, limited insert size and slower onset of gene expression, possibly due to cytoplasmic trafficking, vector uncoating, and conversion of the single-stranded genome into double-stranded DNA. Recent concern has been raised about possible immune reactions to AAV vectors, as well, but the level of reaction has been no where near that observed with adenoviruses.

Retrovirus vectors have been used for a number of gene transfer applications. A principle limitation of these vectors is the need for active cell cycling for integration and expression of the transgene, making these vectors inappropriate for use in end-differentiated tissues like

myocardium. An exception to this limitation is the human immunodeficiency (HIV)-based vectors, also called lentiviral vectors, which have been shown to efficiently transfer genes to post-mitotic cells, including cardiac myocytes. HIV is an enveloped virus with a 9.7-kb single-stranded RNA genome. When compared to similar concentrations of adenoviral vectors, advanced generation lentiviral vectors transduce cardiac myocytes at roughly the same efficiency in vitro and by myocardial injection methods in vivo. Efficiency of lentiviral-gene transfer by in vivo intracoronary perfusion methods has not yet been established. A major advantage of lentiviral vectors is long-term gene expression, possibly related to integration of the transgene into the host genome. The major safety concerns for lentiviral vectors include possibilities of insertional mutagenesis from genomic integration and of wild-type reversion. Modern production techniques for lentiviral vectors have several built-in safeguards to virtually eliminate the possibility of wild-type reversion: the vector is devoid of any wild-type HIV genetic material other than the long terminal repeat segments, and the cell lines used to produce these vectors contain modified and isolated wild-type HIV genes to further reduce the possibility of wild-type reversion. For a more complete discussion of gene transfer vectors, please see a recent review by Lundstrom.

Gene Delivery Methods

Delivery of gene transfer vectors to the large mammalian heart has been shown using myocardial injection, intracoronary perfusion and atrial epicardial gene painting methods. In mice, cardiac uptake after systemic administration via tail vein injection has also been demonstrated, but viability for this technique has never been shown for larger mammals. Several reports have documented effective delivery by direct injection either from the endocardium with catheters or from the epicardium. Direct injection of the vector provides very dense local gene transfer. Quantification of spread from the injection site has shown that gene transfer occurs over a width of only a few millimeters from the needle track. For whole heart delivery, considerable effort has been focused on developing arterial perfusion methods. A series of investigations have shown that the best circumstances for gene transfer included maximal local vasodilation, exposure to permeability-enhancing agents (inflammatory agents, vascular endothelial growth factor, phosphodiesterase 5 inhibitors, nitric oxide or cyclic GMP donors, etc.), and perfusion with the highest tolerable virus concentration for the longest tolerable time. With attention to these details, evidence of gene transfer can be seen in approximately half of cells in the target with antegrade perfusion of the target artery and in more than 80% of cells with simultaneous antegrade and retrograde perfusion of the artery and vein pair (see Sasano et al for a more thorough description of intracoronary perfusion techniques). Of course, retrograde venous perfusion requires identification of the vein draining the target, which is not always possible.

Gene delivery to the atria is considerably more complicated than global ventricular or localized myocardial delivery. Size, geometry and tissue thickness limit utility of myocardial injection methods. The absence of a dedicated atrial vasculature limits intracoronary perfusion methods. To date, the only reported widespread atrial gene transfer method is epicardial gene painting. We showed that complete transmural gene transfer could be achieved by painting a solution containing the vector, a polymerization compound, and dilute protease onto the atrial epicardium. The polymerization compound (pluronic F127) caused the vector to stick to the tissue, increasing contact time and probability of gene transfer. The inclusion of trypsin in the mixture allowed transmural penetration of the vector. In safety testing, it was found that effective concentrations of trypsin were sufficiently dilute so as not to affect atrial structure and tensile strength. Specificity of the technique was 2-fold: the control provided by direct application with a paint brush limited spread beyond the target area, and the ventricular tissue appeared impervious to gene transfer by this method (even when the solution was directly applied to the ventricular

epicardium), suggesting that some intrinsic difference between atrial and ventricular tissue allowed atrial gene transfer but not ventricular gene transfer. With this method, a complete, transmural atrial gene transfer was achieved without evidence of ventricular gene transfer when using the β-galactosidase reporter gene.

Atrial Arrhythmias

Atrial fibrillation is the most common arrhythmia encountered in clinical practice. There are 2-5 million sufferers in the U.S. alone. The most important clinical manifestations of atrial fibrillation are the chaotic electrical activity leading to diminished atrial pump function, and the accompanying fast and irregular ventricular rate. The uncoordinated electrical activity and reduced pumping capacity of atria causes blood to pool in the appendages and increases the likelihood of clot formation, which is the leading cause of stroke. In addition, high ventricular rates can lead to ventricular tachycardiomyopathy, further compounding mortality risk. The current therapeutic strategies are aimed at restoring sinus rhythm or controlling rate. The gene therapy approaches aiming to achieve these clinical objectives are reviewed here.

Control of Atrial Rhythm

One of the major electrophysiological changes which accompanies lone atrial fibrillation is an abbreviation of action potential duration and, therefore, refractory period. This provides the substrate for abbreviated re-entrant wavelengths, which serve to stabilize the chaotic atrial electrical activity. In contrast, the substrate underlying atrial fibrillation in a heart failure setting is thought to be fibrosis. Several alterations of ionic currents have been shown to associate with atrial fibrillation. Recently, our laboratory has successfully targeted atrial electrophysiology to terminate atrial fibrillation. In that study, we exploited the unique properties of KCNH2-G628S mutant channel, a dominant negative mutation that blocks within the ion channel pore region. The methods combined focal atrial painting with Ad.KCNH2-G628s virus to a pig with pacemaker-induced atrial fibrillation. On day 7 of the study (i.e. time of peak transgene expression, post-viral painting) all Ad.KCNH2-G628s-treated animals had returned to sinus rhythm; however, by day 21, all animals regressed into atrial fibrillation with diminished transgene expression levels from the adenovirus vector. Consistent with the lack of atrial fibrillation on day 7, was that the monophasic action potential showed considerable prolongation in Ad.KCNH2-G628s-treated animals at day 7 compared to control animals. This method has excellent translational potential, with the initial patient population most likely to benefit from this approach being those patients undergoing cardiac surgery who would already have epicardial atrial access for the delivery technique.

Levy and colleagues demonstrated the concept of drug-controlled activation of atrial APD prolongation. Porcine atria underwent intraatrial injections of naked DNA plasmids encoding the human MIRP1-Q9E mutant driven by a clarithromycin inducible promoter. MIRP1-Q9E mutations are associated with the Long QT syndrome due to decreased delayed rectifier currents. After clarithromycin administration, site-specific prolongation of the atrial MAP could be recorded. The investigators did not assess the anti-arrhythmic efficacy of this approach. Nonetheless, the choice of target for treatment of atrial fibrillation may be efficacious given the prolongation of MAP.

Rate Control

Atrial fibrillation in most cases produces excessively high ventricular rates and if allowed to persist will likely result in tachycardiomyopathy. One approach to the treatment of these patients is to control the ventricular rate by modulating the only conduit for impulse

propagation to the ventricles, the atrioventricular (AV) node. The focal attributes of gene therapy make this an attractive approach, since the major detriment to pharmacological AV nodal block is off target side effects. We selected the inhibitory G-protein (Gi) to be incorporated into Ad and then transferred to the porcine AV node with the aim of diminishing its excitatory capacity. Adenovirus-mediated gene transfer of the wild type Gi (wtGi) produced an overall 20% reduction of the maximal ventricular rate during acute atrial fibrillation. However, wtGi proved effective only under sedation so we improved this work by incorporating a constitutively active mutant isoform of Gi-Q205L (Ad.cGi), which gave similar rate reduction in awake animals with persistent atrial fibrillation. The translational potential of this approach was significant, but the need is less obvious than the above rhythm control example because pharmacological and ablation procedures are more reliable in the AV node. One important unknown for cGi is the potential effect of inadvertent ventricular gene transfer. The ventricular effects of this gene have not been investigated, but possibilities include a therapeutic effect from an intracellular β-adrenergic block-like action or a detrimental effect from potential negative inotropy.

A novel cell therapy approach for the alteration of AV nodal excitability - although not strictly gene therapy - deserves comment due to the potential for combined gene and cell therapy strategies. Fibroblast cell suspensions were injected into the anterior and posterior approaches of the AV node with the aim of modifying the fast and slow pathways, respectively. Significant increases in AH interval were achieved. However, RR intervals during atrial fibrillation failed to be increased significantly. The retention of exogenous cells at the AV node was a significant achievement. However, there are several concerns to this approach such that a large volume of cell suspension seems to be required for adequate retention of cell numbers. The authors proposed a mechanism involving reduced summation of AV nodal inputs as the cause of AH interval prolongation, however, this wasn't rigorously proven. Alternative explanations for the observed effects could be increased pathlength and/or creation of so-called “dead-end” pathways for wavefront propagation.

Ventricular Arrhythmias

Cardiac Alternans

The presence of beat-to-beat alternation of the cardiac action potential duration is linked to the onset of ventricular arrhythmias and sudden death. The cellular mechanisms underlying these events have been proposed to be either alterations in ionic currents or intracellular calcium cycling, manifested by a steep slope of restitution. Cutler et al., used adenovirus-mediated overexpression of SERCA2a (via aortic cross-clamp method) to increase the rate of diastolic calcium uptake into the sarcoplasmic reticulum. 72 hours after guinea pig hearts had received Ad.SERCA2a, myocytes were isolated. Ad.SERCA2a treated cells demonstrated faster calcium reuptake kinetics and calcium transient amplitude compared to non-infected cells. In addition, Ad.SERCA2a myocytes exhibited a lack of APD and calcium transient alternans during pacing at 200 and 240 bpm when compared to control. Furthermore, in vivo measurements of the threshold (heart rate) for APD alternans was significantly increased in the Ad.SERCA2a treated group. The overall arrhythmogenic efficacy of rapid pacing was significantly reduced in the Ad.SERCA2a group. With the aortic cross-clamp method, the authors achieved a gene transduction efficiency of 50%, but this was largely confined to the epicardial layer. It will be interesting to see if similar efficacy is maintained in a pathological model such as heart failure where alternans is linked to greater arrhythmogenic risk. A major consideration for translation of these findings is the likely need to deliver the transgene to all ventricular myocytes for efficacy.

Post-Myocardial Infarction - Healing Phase

There are numerous remodelling events taking place in the hours and days following a myocardial infarction. Slow conduction is a characteristic feature post-MI and has been attributed to both altered gap junction protein expression and/or reduced upstroke velocity of the action potential. One of the purported mechanisms for reduced upstroke velocity is a more positive resting membrane potential in scar border myocytes. Consequently, there is inactivation of some of the sodium current carried by the Nav1.5 (SCN5A) channel. Rosen and colleagues sought to overcome this by searching for sodium channels with similar overall function yet more positive voltage-dependence of inactivation, which would still maintain a large sodium conductance even at positive membrane potentials. The skeletal muscle sodium channel isoform, SkM1, met those requirements. An initial proof-of-concept was undertaken in a computer generated canine ventricular action potential model. They then incorporated SkM1 into an adenoviral gene delivery system (Ad.SkM1). The sites targeted for Ad.SkM1 injections were those with wide-complex, bipolar epicardial electrograms. Ad.SkM1 group showed improved conduction in silico and narrower fractionated epicardial bipolar signals. Furthermore, significant reductions in ventricular tachyarrhythmias elicited by programmed stimulation were observed. However, in two of the animals in the Ad.SkM1 group the treatment failed to abolish the arrhythmia. This is perhaps due to one or more of the following reasons: mistargeting of the gene transfer (e.g. failure to correctly map the site of arrhythmia initiation); additional regions of slow conduction underlying the arrhythmias being in remote, non-treated regions; resting membrane potential was relatively normal in these animals pre-treatment; Ad.SkM1 enabled propagation through an otherwise non-conducting pathway, or VT continued even in the presence of improved conduction. These are important issues to consider when assessing translational potential.

The majority of gene transfer strategies aiming to modify electrophysiological function have overexpressed a specific gene of interest, with subsequent overexpression of the mRNA and protein. However, microRNAs have emerged as additional regulators of the translational of mRNA into protein. Wang and colleagues, were able to show increased expression of the microRNA, *miR*-1, in post-MI hearts from human and rat. The specific mechanism of increased arrhythmia vulnerability as a result of increased *miR*-1 expression was thought to be due to decreased conduction velocities and/or more positive resting membrane potentials with inhibited expression of GJA1 and KCNJ2. In a rat MI model they targeted *miR*-1 with antagomirs. Twelve hours post-intramyocardial injection, animals treated with the antagomir to *miR*-1 showed suppression of arrhythmia vulnerability; in addition, significantly enhanced conduction velocity and decreased resting membrane potential were observed with antagomir pretreatment.

Post-Myocardial Infarction - Healed Phase

Approximately 4 weeks post-MI, the general healing process (i.e. inflammation, myocyte cell death and cell debris clearance, collagen deposition, etc.) has receded. This substrate therefore represents the large majority of the post-MI patient population at risk of developing ventricular tachyarrhythmias. To best replicate this clinical scenario - in order to study the associated ventricular arrhythmias and develop novel therapies - our laboratory has developed a model of post-MI ventricular tachycardia. Pigs undergo LAD blockade with a balloon catheter for 2.5 hours to create a large anterior MI. After 4-5 weeks there is reproducible VT with programmed stimulation.

Our lab has adopted a strategy to modify the reentrant wavelength underlying ventricular tachycardia in the post-myocardial infarction setting. In initial proof-of-concept studies, we exploited the same dominant-negative KCNH2-G628S mutation described above for AF

therapy. We tested the hypothesis that, localized in vivo gene transfer of this mutant channel to the VT site would prolong the refractory period, thereby extending the reentrant wavelength and eliminating VT circuit formation. We delivered Ad.KCNH2-G628S to the anterior septum via the left anterior descending coronary artery and great cardiac vein. Controls were either no gene transfer or adenovirus containing the beta-galactosidase gene. With this method, 50% of cells of the septal scar border show evidence of transgene expression. One week after gene transfer, the animals receiving AdKCNH2-G628S no longer had any ventricular arrhythmias, but all control animals continued to have inducible sustained monomorphic VT.

The Long QT Syndrome

Brunner et al., utilized gene transfer methods to electrophysiologically reverse a genetically created a Long QT Syndrome variant in transgenic mice. The first transmembrane segment of the rat delayed rectifier current, Kv1.1, together with a HA-tag was incorporated into a mouse genome. The expression of this channel caused native Kv1.5 channels to be retained within the endoplasmic reticulum. This dominant-negative suppression of Kv1.5 currents manifested as a Long QT phenotype. With this model, the author's adenoviral overexpression of Kv1.5 to the base of the LV shortened APD and QT interval. Unfortunately, there was little incidence of arrhythmias in this model at baseline, therefore, inferring potential anti-arrhythmic efficacy of this approach was difficult. This group also observed similar electrophysiological results with the use of an AAV vector six months post-gene transfer.

Bradyarrhythmias

Another common consequence of age-related cardiac electrical dysfunction is diminished ability to maintain an adequate heart rate. In this scenario, the conduction system is the culprit tissue. This may be due to either an inability of the sinus node to drive the atrial tissue at an adequate rate or to impaired atrioventricular conduction. Electronic pacemaker implantation has proven to be an effective method to circumvent this problem but currently lacks the incorporation of sufficient physiological feedback parameters to grant greater freedom to the recipient. Other disadvantages are the mechanical nature of the treatment and associated risks (i.e. lead fracture, infection) compared to a more natural biological approach. There are several major obstacles to be overcome for a biological pacemaker to be able to challenge the electronic version. The robust and dynamic nature of pacemaking is likely to be an inextricably coordinated network of ion/exchange currents, calcium-release events and signalling molecules (amongst others), not one “master” regulator. Some necessary requirements for successful translation are longevity, regularity and adaptation of pacemaking. A number of laboratories are pursuing this complex biological task with vigor and early studies have yielded some interesting results.

One of the original strategies to increase heart rate was to transfer the human β2 adrenoceptor to murine isolated myocytes and in vivo murine right atria. Increased spontaneous activity of isolated myocytes was observed in β2 adrenoreceptor myocytes compared to control, non-transfected myocytes. Furthermore, mice injected with the beta-2 adrenoceptor into their right atria increased their heart rate by approximately 40%. Unfortunately, the authors failed to rigorously explore the specific mechanisms (i.e. increased ionic currents or alterations calcium handling) for the observed increased rate, and the extraordinarily transient duration of the findings (even for plasmid mediated gene transfer) suggest the possibility of either genotoxicity or an unrelated cause for the observed heart rate change.

One of the most simple approaches to generating spontaneous activity in otherwise quiescent cells was by Marban and colleagues who effectively abolished the inward rectifier current, IK,1, which is normally responsible for stabilizing resting membrane potential. Specifically, a dominant negative isoform, Kir2.1-AAA, was packaged into an adenovirus and transferred to guinea pig myocardium using the aortic cross-clamp method. Regular beats of ventricular origin could be recorded on the ECG. Furthermore, it appears the key to obtaining spontaneous depolarization was the degree to which IK,1 could be suppressed: of all the cells that had been successfully transduced, only those with an IK,1 density of <0.4 pA/pF were able to generate spontaneous depolarizations. Given the inhomogeneity of this gene transfer approach and also that these cells exhibited prolongation of APD, it is surprising that multiple regions of ectopic activity (either automatic or EADs) weren't present. Therefore, further investigation into why certain regions were able to drive the surrounding ventricular tissue in that region but not others would be valuable information for possible consideration in future biological pacemaker strategies.

Rosen and colleagues have made considerable effort with regards to biological pacemaking. The general theme of this group has centered around the funny current, I_f, and the exploitation of its molecular composition. Native sinus node I_f is thought to be a heterotetramer composed of HCN2 and HCN4. Although in vitro studies have shown pure populations of either isoform alone is able to reproduce the major I_f characteristics, most notably activation at hyperpolarized membrane potentials. Qu et al., incorporated mouse HCN2 into an adenovirus and injected it into the LA of dogs. Infected cells showed large increases in I_f. Upon vagal nerve stimulation (to suppress native sinus node discharge), earliest electrical activity could be measured from the gene transfer site. In an alternative study they used the same virus but moved the injection site to the posterior portion of the canine LBB and assessed the possibility of improved ventricular escape rhythms. Elimination of native supraventricular rhythms revealed more rapid ventricular escape beats compared to controls. However, the proarrhythmic activity observed up to 48 hours post-injection is concerning with respect to translational potential. HCN1 has also been shown to have similar pacemaking features when injected into porcine atria post-sinus node ablation.

Rosen and colleagues are one of few investigators to successfully combine the two fields of cell therapy with gene transfer for their specific application. They created a novel biopacemaker by electroporating human mesenchymal stem cells with a DNA plasmid encoding HCN2 and then injected approximately 1 million cells into the anterior wall of the canine left ventricle. After ablation of the AV node, 5 of 6 animals demonstrated left ventricular spontaneous activity after 10-12 days and continued for up to 42 days, which is encouraging. The success of the approach appeared to be related to the number of cells injected i.e. more cells injected = greater percentage of cell transplant-driven beats. Pacemapping confirmed the origin of the beats to cell injection sites. Furthermore, the infusion of epinephrine enables increased rate increases of cell-transplant-driven beats although with somewhat variable efficacy. One limitation of the approach is the site of injection not being optimal for replication of the normal ventricular activation sequence, therefore it will be interesting to see if alternative sites would provide more of a native activation sequence.

Future Perspectives

The vast majority of the examples of gene therapy in this review have used a single gene to modify electrophysiological function, in most cases with great effect (table). So far, many of the endpoints have been limited to short timeframes due to the constraints on expression with adenovirus vectors. However, cardiac electrophysiological function under normal circumstances and disease is far from a single gene or molecule process. Therefore, it will be

interesting to see if the examples shown here stand-up to more rigorous preclinical testing at later time-points or if further optimization (as with most novel therapies) is required at any stage of the translational process. One fear with these approaches is the inherent plasticity of biological processes or feedback and, therefore, the potential for compensatory changes in response to the transgene within the system being targeted. If compensatory changes do occur, can they be mitigated with the transfer additional genetic elements i.e. small RNAs to target compensatory gene pathways?

Alternatively, future gene transfer endeavors may find greater efficacy by modifying multiple genes simultaneously. Advances in cloning such as the development of transposon technology will allow the creation of polycistronic transgenes, which could make this a real possibility. Inducible promoters may allow for gene induction only under certain conditions/ stressors i.e. perturbations in cellular microenvironments.

Optimization strategies for re-targeting viruses to the heart alone, specific regions of the heart or even better, diseased-tissue/cells - thereby sparing any potential undesirable consequences of targeting healthy tissue, has received little attention. This is one of the ultimate goals for gene therapy since many diseases are tissue specific yet the target genes are not. Efforts may revolve around targets known to be cardiac exclusive. Alternatively, high-throughput peptide libraries may be an approach to provide novel cardiac targets.

Overall, most current arrhythmia gene therapy studies have been limited duration and “proof-of-concept” in nature. This young field is continually evolving. Current problems that need attention include more efficient and specific delivery to the myocardial target(s), less immunogenic gene transfer vectors, and controllable long-term gene expression.

Gene therapy has recently had some incredible success stories in other fields such as remission from adrenoleukodystrophy, reconstitution of immune function in severe combined immunedeficiency and the restoration of vision in Leber's congenital amaurosis. We believe it is only a matter of time before gene therapy strategies are a viable option for the treatment of cardiac arrhythmias.

References

1. Fox CS, et al. Temporal trends in coronary heart disease mortality and sudden cardiac death from 1950 to 1999: the Framingham Heart Study. Circulation. 2004; 110(5):522–527. [PubMed: 15262842]
2. Echt DS, et al. Mortality and morbidity in patients receiving encainide, flecainide, or placebo. The Cardiac Arrhythmia Suppression Trial. N Engl J Med. 1991; 324(12):781–788. [PubMed: 1900101]
3. Lipskaia L, et al. Sarcoplasmic reticulum Ca(2+) ATPase as a therapeutic target for heart failure. Expert Opin Biol Ther. 2010; 10(1):29–41. [PubMed: 20078230]
4. Kikuchi K, et al. Targeted modification of atrial electrophysiology by homogeneous transmural atrial gene transfer. Circulation. 2005; 111(3):264–270. [PubMed: 15642761]
5. Sasano T, et al. Targeted high-efficiency, homogeneous myocardial gene transfer. J Mol Cell Cardiol. 2007; 42(5):954–961. [PubMed: 17484913]
6. Nattel S, et al. Arrhythmogenic ion-channel remodeling in the heart: heart failure, myocardial infarction, and atrial fibrillation. Physiol Rev. 2007; 87(2):425–456. [PubMed: 17429037]
7. Lin H, et al. Expression of recombinant genes in the myocardium in vivo after direct injection of DNA. Circulation. 1990; 82(6):2217–2221. [PubMed: 2173647]
8. Shenk, T. Fields of Virology. 3rd. Philadelphia: Lippincott-Raven Publishers; 2010. Adenoviridae: the viruses and their replication; p. 2111-2148.
9. Ferrari FK, et al. Second-strand synthesis is a rate-limiting step for efficient transduction by recombinant adeno-associated virus vectors. J Virol. 1996; 70(5):3227–3234. [PubMed: 8627803]

10. Hauck B, et al. Intracellular viral processing, not single-stranded DNA accumulation, is crucial for recombinant adeno-associated virus transduction. J Virol. 2004; 78(24):13678–13686. [PubMed: 15564477]
11. High K, et al. Human immune responses to AAV-2 capsid may limit duration of expression in liver-directed gene transfer in humans with hemophilia B. Blood. 2004; 104(11):413.
12. Cleghorn, FR., et al. Human immunodeficiency viruses. In: Mandell, GL.; Bennett, JE.; Dolin, R., editors. Principles and Practice of Infectious Diseases. 2010. p. 2119-2130.
13. Bonci D, et al. 'Advanced' generation lentiviruses as efficient vectors for cardiomyocyte gene transduction in vitro and in vivo. Gene Ther. 2003; 10(8):630–636. [PubMed: 12692591]
14. Cockrell AS, Kafri T. Gene delivery by lentivirus vectors. Mol Biotechnol. 2007; 36(3):184–204. [PubMed: 17873406]
15. Lundstrom K. Latest development in viral vectors for gene therapy. Trends Biotechnol. 2003; 21(3):117–122. [PubMed: 12628368]
16. Gregorevic P, et al. Systemic delivery of genes to striated muscles using adeno-associated viral vectors. Nat Med. 2004; 10(8):828–834. [PubMed: 15273747]
17. Kass-Eisler A, et al. Quantitative determination of adenovirus-mediated gene delivery to rat cardiac myocytes *in vitro* and *in vivo*. Proc Natl Acad Sci USA. 1993; 90(12):11498–11502. [PubMed: 8265580]
18. Benjamin EJ, et al. Impact of atrial fibrillation on the risk of death: the Framingham Heart Study. Circulation. 1998; 98(10):946–952. [PubMed: 9737513]
19. Amit G, et al. Selective Molecular Potassium Channel Blockade Prevents Atrial Fibrillation. Circulation. 2010
20. Curran ME, et al. A molecular basis for cardiac arrhythmia: HERG mutations cause long QT syndrome. Cell. 1995; 80(5):795–803. [PubMed: 7889573]
21. Burton DY, et al. The incorporation of an ion channel gene mutation associated with the long QT syndrome (Q9E-hMiRP1) in a plasmid vector for site-specific arrhythmia gene therapy: in vitro and in vivo feasibility studies. Hum Gene Ther. 2003; 14(9):907–922. [PubMed: 12828861]
22. Abbott GW, et al. MiRP1 forms IKr potassium channels with HERG and is associated with cardiac arrhythmia. Cell. 1999; 97(2):175–187. [PubMed: 10219239]
23. Donahue JK, et al. Focal modification of electrical conduction in the heart by viral gene transfer. Nat Med. 2000; 6(12):1395–1398. [PubMed: 11100126]
24. Bauer A, et al. Inhibitory G protein overexpression provides physiologically relevant heart rate control in persistent atrial fibrillation. Circulation. 2004; 110(19):3115–3120. [PubMed: 15505099]
25. Bunch TJ, et al. Impact of transforming growth factor-beta1 on atrioventricular node conduction modification by injected autologous fibroblasts in the canine heart. Circulation. 2006; 113(21): 2485–2494. [PubMed: 16717152]
26. Rosenbaum DS, et al. Electrical alternans and vulnerability to ventricular arrhythmias. N Engl J Med. 1994; 330(4):235–241. [PubMed: 8272084]
27. Cutler MJ, et al. Targeted SERCA2a gene expression identifies molecular mechanism and therapeutic target for arrhythmogenic cardiac alternans. Circ Arrhythm Electrophysiol. 2009; 2(6): 686–694. [PubMed: 19948504]
28. Peters NS, et al. Disturbed connexin43 gap junction distribution correlates with the location of reentrant circuits in the epicardial border zone of healing canine infarcts that cause ventricular tachycardia. Circulation. 1997; 95(4):988–996. [PubMed: 9054762]
29. Lau DH, et al. Epicardial border zone overexpression of skeletal muscle sodium channel SkM1 normalizes activation, preserves conduction, and suppresses ventricular arrhythmia: an in silico, in vivo, in vitro study. Circulation. 2009; 119(1):19–27. [PubMed: 19103989]
30. Yang B, et al. The muscle-specific microRNA miR-1 regulates cardiac arrhythmogenic potential by targeting GJA1 and KCNJ2. Nat Med. 2007; 13(4):486–491. [PubMed: 17401374]
31. Fishbein MC, Maclean D, Maroko PR. Experimental myocardial infarction in the rat: qualitative and quantitative changes during pathologic evolution. Am J Pathol. 1978; 90(1):57–70. [PubMed: 619696]

32. Sasano T, et al. Molecular ablation of ventricular tachycardia after myocardial infarction. Nat Med. 2006; 12(11):1256–1258. [PubMed: 17072309]
33. Brunner M, et al. In vivo gene transfer of Kv1.5 normalizes action potential duration and shortens QT interval in mice with long QT phenotype. Am J Physiol Heart Circ Physiol. 2003; 285(1):H194–H203. [PubMed: 12793978]
34. Kodirov SA, et al. Long-term restitution of 4-aminopyridine-sensitive currents in Kv1DN ventricular myocytes using adeno-associated virus-mediated delivery of Kv1.5. FEBS Lett. 2003; 550(1-3):74–78. [PubMed: 12935889]
35. Edelberg JM, Aird WC, Rosenberg RD. Enhancement of murine cardiac chronotropy by the molecular transfer of the human beta2 adrenergic receptor cDNA. J Clin Invest. 1998; 101(2):337–343. [PubMed: 9435305]
36. Miake J, Marban E, Nuss HB. Biological pacemaker created by gene transfer. Nature. 2002; 419(6903):132–133. [PubMed: 12226654]
37. Qu J, et al. Expression and function of a biological pacemaker in canine heart. Circulation. 2003; 107(8):1106–1109. [PubMed: 12615786]
38. Plotnikov AN, et al. Biological pacemaker implanted in canine left bundle branch provides ventricular escape rhythms that have physiologically acceptable rates. Circulation. 2004; 109(4): 506–512. [PubMed: 14734518]
39. Tse HF, et al. Bioartificial sinus node constructed via in vivo gene transfer of an engineered pacemaker HCN Channel reduces the dependence on electronic pacemaker in a sick-sinus syndrome model. Circulation. 2006; 114(10):1000–1011. [PubMed: 16923751]
40. Plotnikov AN, et al. Xenografted adult human mesenchymal stem cells provide a platform for sustained biological pacemaker function in canine heart. Circulation. 2007; 116(7):706–713. [PubMed: 17646577]
41. Kahlig KM, et al. Multiplexed transposon-mediated stable gene transfer in human cells. Proc Natl Acad Sci U S A. 2010; 107(4):1343–1348. [PubMed: 20080581]
42. Ying Y, et al. Heart-targeted adeno-associated viral vectors selected by in vivo biopanning of a random viral display peptide library. Gene Ther. 2010
43. Cartier N, et al. Hematopoietic stem cell gene therapy with a lentiviral vector in X-linked adrenoleukodystrophy. Science. 2009; 326(5954):818–823. [PubMed: 19892975]
44. Aiuti A, et al. Gene therapy for immunodeficiency due to adenosine deaminase deficiency. N Engl J Med. 2009; 360(5):447–458. [PubMed: 19179314]
45. Bainbridge JW, et al. Effect of gene therapy on visual function in Leber's congenital amaurosis. N Engl J Med. 2008; 358(21):2231–2239. [PubMed: 18441371]

Table 1

Gene therapy strategies for cardiac electrical dysfunction.

Model/application	Gene/target	Vector/vehicle	Delivery method	Target tissue	Species	Result	Reference
Atrial fibrillation	*KCNH2-G628S*	Adenovirus serotype 5	Atrial Painting	Atria	Swine	Restoration of sinus rhythm	[19]
Atrial electrophysiology	*MIRP1-Q9E*	DNA/plasmid	Intramyocardial Injection	RA	Swine	Prolongation of MAP	[21]
Acute atrial fibrillation	*wtGi*	Adenovirus serotype 5	Intracoronary	AV node	Swine	~20% reduction in maximum ventricular rate	[23]
Persistent atrial fibrillation	*cGi*	Adenovirus serotype 5	Intracoronary	AV node	Swine	~20% reduction in maximum ventricular rate	[24]
Post-myocardial infarction ventricular tachycardia	*SkM1*	Adenovirus serotype 5	Intramyocardial Injection	Anterior LV	Canine	Significant reductions in ventricular tachyarrhythmias	[29]
Post-myocardial infarction ventricular tachyarrhythmias	*miR-1*	Small RNAs	Intramyocardial Injection	LV	Rat	Increased conduction velocity; reduced ventricular tachyarrhythmias	[30]
Post-myocardial infarction ventricular tachycardia	*KCNH2-G628S*	Adenovirus serotype 5	Intracoronary	Anterior septal LV	Swine	Abolition of ventricular tachyarrhythmias	[32]
Long QT	*Kv1.5*	Adenovirus serotype 5 and adeno-associated virus	Intracoronary	LV	Mouse	Abbreviated APD and QT interval	[33,34]
Cardiac alternans	*SERCA2a*	Adenovirus serotype 5	Aortic cross-clamp	LV	Guinea Pig	Decreased alternans susceptibility and arrhythmia vulnerability	[27]
Biopacemaking	*Kir2.1-AAA*	Adenovirus serotype 5	Aortic cross-clamp	Basal LV	Guinea Pig	Pacemaking in vitro; regular and catecholamine responsive pacemaking in vivo	[36]
Biopacemaking	*β2-adrenoceptor*	DNA/Plasmid	Intramyocardial Injection	RA	Mouse	Increased spontaneous activity in vitro; 40% increase in resting HR	[35]
Biopacemaking	*HCN2*	Adenovirus serotype 5	Intramyocardial Injection	LA	Canine	Ability to independently drive atrial tissue	[37]
Biopacemaking	*HCN1-ΔΔΔ*	Adenovirus serotype 5	Intramyocardial Injection	LA	Swine	Ability to independently drive atrial tissue	[39]
Biopacemaking	*HCN2*	DNA plasmid+ mesenchymal stem cells	Intramyocardial Injection	Anterior LV	Canine	Ability to independently drive ventricular tissue; catecholamine responsive	[40]