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Gene therapy to restore electrophysiological function in heart failure

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Abstract

Introduction—Heart failure (HF) is a major public health epidemic and a leading cause of morbidity and mortality in the industrialized world. Existing treatments for patients with HF are often associated with pro-arrhythmic activity and risk of sudden cardiac death (SCD). Therefore, development of novel, effective, and safe therapeutic options for HF patients is a critical area of unmet need.

Areas Covered—In this article, we review recent advances in the emerging field of cardiac gene therapy for the treatment of tachy- and brady- arrhythmias in HF. We provide an overview of gene-based approaches that modulate myocardial conduction, repolarization, calcium cycling, and adrenergic signaling to restore heart rate and rhythm.

Expert Opinion—We highlight major advantages of gene therapy for arrhythmias, including the ability to selectively target specific cell populations and to limit the therapeutic effect to the region that requires modification. We illustrate how advances in our fundamental understanding of the molecular origins of arrhythmogenic disorders are allowing investigators to use targeted gene-based approaches to successfully correct abnormal excitability in the atria, ventricles, and conduction system. Translation of various gene therapy approaches to humans may revolutionize our ability to combat lethal arrhythmias in HF patients.

Keywords

Heart Failure; Arrhythmias; Conduction; Repolarization; Calcium; Gene Therapy

1. INTRODUCTION

Heart failure (HF) is a major public health epidemic worldwide [1]. Arrhythmogenic disorders account for a significant portion of HF-related morbidity and mortality [2]. Our incomplete understanding of fundamental arrhythmia mechanisms in the failing heart has hampered the development of effective and safe pharmacological treatments [3]. In fact, the

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pro-arrhythmic tendency of some pharmacological agents has resulted in the early termination of the Cardiac Arrhythmia Suppression Trial (CAST) after a substantial increase in arrhythmic deaths was noted in post-myocardial infarction (MI) patients treated with encanide or flecainide compared to placebo [4].

HF leads to a wide variety of arrhythmic disorders. Bradyarrhythmias arise from defects in one or more elements of the cardiac conduction system, such as the sinoatrial node (SAN) or the atrioventricular node (AVN). These defects manifest as sick sinus syndrome or heart block. If extreme, bradyarrhythmias can lead to cardiac insufficiency and death. More commonly, slow, irregular, and abnormal ventricular activation patterns in bradyarrhythmic patients promote adverse ventricular remodeling, hypertrophy, and disease progression. This, in turn, heightens their propensity for SCD. On the other hand, tachyarrhythmias in HF are classified as ventricular or supraventricular depending on whether they originate in the ventricular chambers (VT) or elsewhere (SVT). Of note, atrial fibrillation (AF), the most prevalent arrhythmic disorder in humans, is a common form of SVT that is closely intertwined with HF.

Tachyarrhythmias are caused by enhanced automaticity, triggered activity, and/or reentry [5]. The first two are cellular phenomena whereas the latter is a property of the cardiac syncytium. Enhanced automaticity describes the acceleration of the spontaneous firing rate of action potentials. When the automaticity of ventricular myocytes is elevated in response to disease, irregular activation patterns can develop. On the other hand, triggered activity refers to calcium (Ca^{2+})-mediated premature action potentials that arise from so-called early or delayed after depolarizations (EADs and DADs, respectively). Finally, reentry is the most common tachyarrhythmia mechanism in HF. This is a multi-cellular process involving one or more excitation wavefronts that propagate around zones of refractory tissue or rotate as spiral waves.

In recent years our fundamental understanding of the molecular origins of arrhythmogenic disorders has expanded considerably. This has allowed investigators to use targeted gene-based approaches to correct abnormal excitability in the atria, ventricles, and the cardiac conduction system. In this article, we provide an update on some of the most promising gene therapy approaches that have emerged in recent years for the treatment of tachy- and brady-arrhythmias in HF [6]. Details including the target gene, choice of vector, delivery technique, target tissue, and species are shown in Tables 1–3. Classification of various approaches according to their stage of development and translation is illustrated in Table 4.

2.1. GENE THERAPY FOR VENTRICUALR TACHYCARDIA IN HEART FAILURE

Patients with HF are highly prone to develop malignant episodes of VT that culminate in SCD [2]. Unfortunately, antiarrhythmic drug therapy in this patient population often increases or at best has a neutral effect on cardiac-related mortality [4, 7–11]. While device therapies (namely, implantable cardioverter defibrillators, ICDs) are effective, they do not cure the arrhythmogenic disorder. Instead, ICDs are designed to convert tachyarrhythmias following their onset [12]. As such, these devices neither treat the root cause of the disease nor prevent its imminent recurrence. Moreover, inappropriate ICD-mediated shocks can substantially reduce patients' quality of life by causing a variety of psychopathological

disorders [12]. To add insult to injury, ICD implantation is associated with frequent surgical complications as well as device and lead failures [13]. Hence, there is a major unmet need for novel, mechanism-based, and curative approaches for VT in HF. Clearly, such treatments require a deep understanding of the molecular, cellular, and tissue-level remodeling that causes VT in the failing heart. Collectively, this remodeling promotes the formation of arrhythmogenic triggers and a malignant substrate amenable for sustaining VT. Here, we highlight recent efforts to combat VT using gene-based approaches designed to restore repolarization, conduction, and intracellular Ca^{2+} cycling in the failing heart (Table 1).

2.1.1. Gene therapy to restore abnormal repolarization in HF—An electrophysiological hallmark of the failing heart is a prolonged action potential duration (APD), reflecting delayed terminal repolarization of the cardiac myocyte [2, 14]. Excessive APD prolongation in HF facilitates the formation of EADs. The ionic mechanism involves the recovery of the L-type calcium current ($I_{Ca\text{-}L}$) from inactivation during the prolonged duration of the action potential [15, 16]. This, in turn, results in the voltage-dependent reactivation of $I_{Ca\text{-}L}$ at a time when membrane resistance is high (plateau phase). $I_{Ca\text{-}L}$ reactivation results in a secondary membrane depolarization that interrupts the repolarization phase of the previous beat. If large enough, this secondary depolarization can reach the threshold required to propagate to downstream cells forming a premature beat. Importantly, EAD-mediated premature beats that arise during the so-called “vulnerable window” of repolarization can initiate sustained arrhythmias via a reentrant mechanism [17]. For this to happen, an electrophysiological substrate that facilitates the formation of unidirectional conduction block of the premature impulse is required. Such a substrate could involve electrophysiological changes in the gradients of repolarization and conduction as well as structural discontinuities (i.e. fibrosis, scar), all of which are present in the failing heart. We and others have highlighted the importance of spatially heterogeneous APD prolongation across the ventricular wall in the formation of an arrhythmogenic substrate [18]. In addition, the Rosenbaum group has advanced our understanding of the role of temporal repolarization instabilities, namely repolarization alternans, in the genesis of polymorphic VT in HF [19].

Given the pathophysiological relevance of excessive APD, strategies aimed at normalizing APD in HF are expected to exert protective electrophysiological effects. Unfortunately, available pharmacological agents that shorten APD, such as Ca^{2+} channel blockers, are not ideal in certain situations because of their confounding effects on contractility or blood pressure in a patient population that often requires inotropic support [20]. As such, novel gene therapies that normalize APD without impairing contractility are warranted. In a proof-of-principal study, Murata and colleagues created a biological Ca^{2+} channel blocker by overexpressing the ras-related small G-protein, Gem [21]. Indeed, adenoviral-mediated delivery of Gem decreased $I_{Ca\text{-}L}$ and shortened APD. As expected, this strategy also blunted contractile function, an effect which limited its utility in end-stage failing hearts [21]. In a follow-up study, Cingolani et al demonstrated that *in vitro* and *in vivo* knockdown of the L-type Ca^{2+} channel β subunit using a short hairpin RNA template sequence reduced $I_{Ca\text{-}L}$ and attenuated the hypertrophic response without compromising systolic performance [22]. Strategies designed to genetically modulate Ca^{2+} channel subunits selectively in the heart could, therefore, represent a useful therapeutic strategy that complements existing Ca^{2+}

channel blockers. These biological strategies have the potential to circumvent the inherent limitations of pharmacological Ca^{2+} channel blockers, including their potent hypotensive effects. By targeting the biological therapy selectively to myocytes using appropriate vectors and promoters, confounding effects on other cell types, such as smooth muscle cells, could be readily avoided.

APD shortening can be achieved either by reducing depolarizing currents (such as $I_{Ca\text{-}L}$) or increasing the activity of repolarizing K channels. The latter strategy was attempted by Lebeche and colleagues [23] who found that adenoviral-mediated overexpression of Kv4.3, the gene encoding I_{to}, prevented pressure overload-induced APD prolongation and, in doing so, abrogated the hypertrophic response via a calcineurin-mediated pathway [23]. While I_{to} does not play a primary role in the control of terminal repolarization in humans or large animals, it sets the plateau voltage at which all subsequent currents are activated. Thus, it will be critical to evaluate the impact of this strategy in more clinically-relevant animal models. A major issue to be resolved is the effect of I_{to} overexpression on the activation profile of $I_{Ca\text{-}L}$, which may prolong rather than shorten APD. Moreover, I_{to} is heterogeneously expressed across the ventricular wall with epicardial cells exhibiting the greatest magnitude [24]. It will be important to evaluate whether these ionic heterogeneities, which have been implicated in arrhythmic disorders such as the Brugada Syndrome [25], are elevated or reduced following gene transfer of Kv4.3. Nonetheless, the findings of Lebeche and colleagues imply that K channel remodeling may not simply be a consequence of HF but rather a cause for it. This highlights the potential importance of normalizing APD as a strategy for hindering disease progression. Whether gene-based approaches aimed at overexpressing repolarizing K channels can reverse-remodel the failing heart following onset of left ventricular (LV) dysfunction is yet to be determined.

Unlike in rodents, terminal repolarization in humans and large animals is highly dependent on delayed rectified K channels [26]. Downregulation of these channels promotes the incidence of polymorphic VT [26]. Mazhari et al [27] used gene transfer to increase the activity of an accessory K channel subunit (KCNE3), a key regulatory component of the delayed rectifier K channel, in a model of LQTS. In doing so, they accelerated cardiac repolarization and abbreviated the QT-interval [27]. Of note, these authors demonstrated using computer modeling that gene delivery strategies targeting K channels could exacerbate arrhythmias if they were to promote repolarization heterogeneity across the transmural wall. This finding reaffirms our earlier observations regarding the functional significance of these heterogeneities [18]. Because HF is in essence an acquired form of LQTS, the findings of Mazhari et al may have broad implications.

2.1.2. Altered Conduction Predisposes to VT in HF—Conduction slowing promotes the initiation and maintenance of reentrant circuits underlying VT in HF. Mechanisms underlying myocardial conduction slowing include reduced myocyte excitability as well as changes in intra-, extra-, and inter-cellular resistivities [28]. Numerous studies have documented the importance of changes in the expression, phosphorylation, and localization of the main ventricular gap junction protein, Cx43 in HF [28–30]. Owing to the importance of Cx43 in the regulation of cell-to-cell coupling, interventions targeting Cx43 expression and/or function may have profound implications for the treatment of HF [31]. In a

preclinical porcine model of post-MI remodeling caused by transient left anterior descending coronary artery (LAD) occlusion, Donahue and colleagues elegantly demonstrated that adenoviral-mediated gene delivery of Cx43 markedly improved conduction velocity and reduced the susceptibility of animals to VT [32]. These findings highlight Cx43 gene delivery as a potential therapeutic strategy for post-MI arrhythmias [32]. Before this strategy can be translated further, however, important safety questions must be addressed. For one, loss of Cx43 in response to ischemic injury hinders the spread of inflammatory mediators. Indeed, Kanno *et al* [33] elegantly demonstrated that Cx43 is a major determinant of MI size. Specifically, they found that Cx43-deficient mice exhibited smaller infarcts in response to coronary occlusion compared to wildtype animals [33]. These authors concluded that therapies designed to suppress arrhythmias by enhancing inter-cellular communication could ultimately lead to larger infarcts [33], and by extension, greater remodeling and more arrhythmias. Indeed, the Rosen group provided direct proof of this principal. They elegantly demonstrated that increased cell-to-cell coupling by gene transfer of the liver specific, pH- and voltage-independent Cx32 isoform to the heart increased infarct size and failed to protect against the incidence of VT in mice [34] and dogs [35].

The posttranslational modification (namely by phosphorylation) of gap junction proteins is critical in maintaining proper cell-to-cell coupling [36]. Whether exogenously introduced Cx43 via gene transfer will retain a favorable phosphorylation state and will exhibit effective forward trafficking, insertion, and stability at the intercalated disk remains unknown. As we gain a comprehensive understanding of the structure-function relationships that govern gap junction communication, we will be able to custom-engineer Cx43 mutants or partner molecules to optimize gene-based therapies for specific HF etiologies [31].

Impulse conduction is highly dependent on the fast inward sodium current (I_{Na}), which has multiple isoforms. Of note, the skeletal muscle isoform exhibits a depolarizing shift in inactivation and a more favorable recovery from inactivation rendering it more resistant to ischemia compared to its cardiac counterpart. In elegant proof-of-principal studies, Protas *et al* [37] showed that gene delivery of the skeletal but not cardiac isoform into the heart was effective in preserving conduction and reducing *in vitro* arrhythmias that arise in the setting of partial membrane depolarization (i.e. simulating ischemia). The utility of this strategy was later extended to a clinically-relevant model of arrhythmias arising at the MI border zone [38]. The studies by Protas *et al* [37] and Lau *et al* [38] emphasize the notion that delivery of non-cardiac genes may be advantageous under conditions in which the native cardiac isoforms are adversely affected. The electrophysiological consequences of increasing I_{Na} density in HF require extensive investigation in the future. Since upregulation of the late component of the current promotes EAD formation, caution must be exercised when overexpressing Na channels.

2.1.3. Reentrant activation underlying VT in HF—The majority of clinical VT cases are maintained by reentrant circuits around an anatomical scar (anatomical reentry) or a zone of refractory tissue (functional reentry) [39]. For reentry to occur, the cardiac wavelength (λ) of the reentrant circuit or the spatial extent of the refractory tail must be shorter than the path-length around which the wavefront circulates. As such, strategies aimed at extending λ in the critical zone where reentrant circuits anchor (i.e. MI border zone) are expected to

suppress the incidence and sustenance of VT. Theoretically, this can be achieved by hastening conduction velocity (extending the wave head) or prolonging local refractoriness (extending the wave tail). In either case, head-tail interactions would destabilize the VT circuit and extinguish reentry. In a landmark study, Donahue and colleagues developed a porcine model of inducible VT originating in the region bordering the healed MI scar. They found that gene transfer of a mutant form (G628S) of the KCNH2 gene which acts as a dominant negative suppressor of the rapidly activating component of the delayed rectifier K current exclusively to the MI border zone resulted in local prolongation of refractoriness [40]. This, in turn, suppressed the incidence of VT by extinguishing the reentrant circuit around the scar [40]. This elegant study highlights a major advantage of targeted gene transfer techniques over existing pharmacotherapies that prolong refractoriness globally and that are known to promote rather than suppress arrhythmias (i.e. some class III drugs) [41]. Specifically, the ability to limit the effect of the therapeutic intervention to precisely the region that required modification (i.e. infarct border) was a key factor in the success of the gene therapy strategy [40]. Finally, these findings highlight the importance of uncovering the tissue-level electrophysiological mechanisms that underlie an arrhythmia before gene therapies can be successfully applied. Unlike non-ischemic dilated cardiomyopathy in which efforts to shorten and homogenize APD are warranted [18], in the case of post-MI arrhythmias local prolongation of refractoriness was effective in eradicating the reentrant rhythm. By mapping the reentrant circuit and defining the critical isthmus, one can envision gene delivery strategies that are targeted exclusively to discrete zones.

2.1.4. Abnormal Calcium Cycling Promotes VT in HF—Defective intracellular Ca^{2+} cycling results in electromechanical dysfunction and arrhythmias in the failing heart [14]. Of particular importance are increased diastolic Ca^{2+} leak from the sarcoplasmic reticulum (SR) via ryanodine receptors (RYR2) and decreased SR Ca^{2+} reuptake by SERCA2a. These two factors promote cytosolic Ca^{2+} overload and a host of metabolic and electrophysiological abnormalities that render the heart susceptible to VT. While SR Ca^{2+} leak promotes DADs and triggered activity, impaired SR Ca^{2+} reuptake via SERCA2a is causally related to the genesis of beat-to-beat repolarization alternans which promote reentrant excitation. In what follows, we highlight a strategy designed to ameliorate HF-related arrhythmias by increasing SERCA2a expression. Our intention is not to comprehensively review all the laudable efforts in this area, but rather to illustrate how targeting defective Ca^{2+} cycling may have therapeutic implications for arrhythmias in the failing heart.

SERCA2a downregulation in HF causes major defects in excitation-contraction coupling [42]. As such, increasing the expression levels of SERCA2a is expected to ameliorate HF-related dysfunction. Early experimental studies using adenoviral-mediated gene transfer have demonstrated the potential for restoring contractile function at no metabolic cost in normal and failing hearts [43]. Targeted gene transfer techniques to increase the expression levels of SERCA2a using adeno-associated viral (AAV) vectors have also been developed, and multicenter trials in humans have been completed [44–46]. The Calcium Up-Regulation by Percutaneous Administration of Gene Therapy in Cardiac Disease (CUPID) trial evaluated the safety profile of SERCA2a gene transfer using AAV serotype 1

(AAV1.SERCA2a) in patients with advanced HF [45, 46]. Participants were administered a single intracoronary infusion of AAV1.SERCA2a in an open-label approach. 12-month follow-up of patients revealed an acceptable safety profile and improved clinical status [45, 46]. In a subsequent phase 2 trial, 39 patients with advanced HF were randomized to receive intracoronary infusion of AAV1.SERCA2a at low, intermediate, and high doses versus placebo [45, 46]. AAV1.SERCA2a treated patients exhibited improvement or stabilization of their HF status compared to their placebo-treated counterparts [45, 46]. Significant delays in the time to adjudicated cardiovascular events, and a decreased frequency of these events were observed in patients receiving AAV1.SERCA2a. Finally, AAV1.SERCA2a-treated patients did not exhibit an increase in adverse events or laboratory abnormalities compared to their placebo counterparts [45, 46].

Despite these highly encouraging findings, it remained unclear whether accelerating SR Ca^{2+} cycling using gene therapy could alter the electrophysiological substrate and the frequency of arrhythmic triggers, an area of major interest considering the general dogma that SR Ca^{2+} overload may exacerbate SR Ca^{2+} leak through RYR2. Indeed, cAMP-dependent PKA phosphorylation which hastens Ca^{2+} cycling, reduces the threshold for Ca^{2+} sparks by enhancing the response of RYR2 to luminal Ca^{2+}. Recent experimental studies in SERCA2a overexpressing hearts, however, have not revealed an increase in arrhythmia susceptibility [47–52]. In a rat model of post-MI remodeling, SERCA2a gene therapy stabilized SR Ca^{2+} load, reduced RyR2 phosphorylation, and decreased SR Ca^{2+} leak [49]. More recently, Cutler and colleagues showed that repolarization alternans were significantly reduced by gene transfer of SERCA2a to guinea pig hearts with systolic HF [48]. Because of the mechanistic link between spatially discordant repolarization alternans and the genesis of ventricular fibrillation, these findings may have important therapeutic implications.

2.2. GENE THERAPY FOR SUPRAVENTRICULAR TACYARRHYTHMIAS IN HEART FAILURE

The rate of firing of action potentials in the SAN is tightly regulated by the autonomic nervous system. It, therefore, follows that the hyperadrenergic state of the failing heart is associated with elevated heart rates. Mechanistically, sympathetic stimulation activates β_1-adrenergic receptors which are coupled to stimulatory G proteins (G_s). Activation of the $G\alpha_s$-subunit stimulates adenyl cylase (AC) and increases intracellular cAMP levels. This, in turn, provokes a cascade of events that culminate in increased chronotropy, dromotropy and inotropy. This stimulatory process is counterbalanced by inhibitory G proteins (G_i), which are coupled to muscarinic and adenosine receptors [53].

The pathophysiological significance of increased heart rate in HF is supported by multiple lines of evidence. For one, a strong association between average heart rate and cardiovascular morbidity is well documented in patients with HF [54, 55]. In clinical trials, the ability of a given HF treatment strategy to reduce overall mortality was often predicted by its heart rate lowering efficacy [54]. Moreover, a causal relationship between elevated heart rate and HF is epitomized by the fact that sustained tachycardia, in the absence of other risk factors, is a well-established mechanism of ventricular remodeling that culminates in HF [56]. Numerous groups have leveraged the relationship between elevated heart rate and LV dysfunction to develop reproducible small and large animal models of HF using chronic

tachycardia-pacing [57]. These experimental models have been instrumental in advancing our fundamental understanding of mechanisms underlying mechanical, structural, electrical, metabolic, ionic, and molecular dysfunction in the failing heart. A chronically elevated ventricular response rate to AF predisposes to the development of HF [58]. This further highlights the role of ventricular rate control in the management of HF symptoms that arise from sinus tachycardia or AF.

2.2.1. Gene therapy for Rate Control: Development of Biological Beta-Blockers —Beta-adrenergic receptor blockers (Beta-blockers) are a mainstay of HF therapy [59]. Indeed, beta-blockers are distinguished by their heart rate lowering efficacy and their ability to hinder and/or reverse key features of pathological remodeling in the failing heart [59]. Because beta-blockers are confounded by negative inotropic properties, their efficacy in the acute phase is often limited. Moreover, the use of beta-blockers is often hampered by their propensity to lower blood pressure as well as other side effects, including depression and erectile dysfunction. In addition, beta-blockers are contraindicated in the presence of diverse comorbidities such as bronchial asthma. To that end, considerable interest has been directed towards the development of biological alternatives to beta-blockers that can achieve adequate heart rate control while avoiding the cardiac and extra-cardiac toxicities of pharmacological beta-blockers. Using an intracoronary approach, Donahue and colleagues delivered an adenovirus carrying the inhibitory $G\alpha_{i2}$-subunit to the porcine AVN. In doing so, they prolonged atrioventricular conduction and reduced the ventricular response rate to paroxysmal AF by 20% [60]. The same approach was also tested in a pig model of persistent AF, in which gene transfer of $G\alpha_{i2}$ also decreased heart rate and normalized ejection fraction [61]. Of note, these authors elegantly reported similar benefits after silencing $G\alpha_s$ in the AVN [62]. More recently, Lungenbiel and colleagues evaluated the efficacy of $G\alpha_s$ silencing in reducing heart rate, when targeted to the SAN rather than the AVN. In the absence of adverse effects on ventricular function, they observed a 16.5% reduction in heart rate and a trend towards diminished sensitivity to isoproterenol [63]. In essence, these authors developed a biological alternative to pharmacological blockers of HCN which have shown significant promise in both preclinical studies and clinical trials [54].

2.2.2. Gene therapy for Rhythm Control in AF—AF, the most common SVT in humans, markedly increases the risk of patients for stroke and HF [58, 64]. Conversely, HF is the strongest risk factor for developing AF [58]. Traditional therapeutic approaches for AF include the use of ablation techniques and pharmacological agents [65]. The goal of the former strategy is to convert AF by burning or freezing foci of arrhythmogenic triggers or imposing anatomical barriers that disrupt the reentrant circuits underlying AF [65]. The progressive nature of AF warrants early device intervention to ensure successful ablation of the arrhythmia. On the other hand, pharmacotherapies for AF are designed to either achieve ventricular rate control (reviewed above in the case of beta-blockers) or rhythm control (i.e. eradicate the arrhythmia) [65]. Unlike ablation techniques, pharmacological agents provide the opportunity to tailor therapy according to the specific pathophysiological processes that underlie the arrhythmia, while avoiding the destruction of atrial tissue by radiofrequency energy or cryoablation. Unfortunately, pharmacological therapies for AF are often severely limited by suboptimal efficacy and a substantial risk of ventricular proarrhythmia [4, 41]. As

such, novel gene-based approaches for AF management in the setting of HF are highly desirable.

At the tissue level, AF is most commonly sustained by circuits of reentrant excitation which are provoked by shortening of atrial repolarization and/or slowing of conduction velocity [66]. Notable efforts have been directed towards normalizing atrial repolarization by suppressing the rapidly activating component of the delayed rectifier K current which is encoded by the human ether-go-go-related gene (HERG). Specifically, Amit et al [67] used atrial gene painting to focally overexpress a dominant negative mutant construct (G628S) of the HERG channel. Their adenoviral-mediated approach, which caused significant prolongation of the atrial action potential duration, was highly effective in suppressing burst-pacing induced AF in a porcine model. A similar strategy was subsequently adopted by Soucek et al [66] who demonstrated comparable findings using a hybrid technique of local viral injection and epicardial electroporation to increase transgene expression in the atria. Since HERG is widely expressed in the ventricles, the success of this strategy is predicated on the ability to modulate atrial but not ventricular properties in order to avoid the formation of torsade de pointes as a result of prolonged ventricular repolarization. Indeed, both Amit et al [67] and Soucek et al [66] achieved atrial-specific gene transfer using two independent methods.

Reentrant excitation underlying AF can also be prevented by improving atrial conduction properties, which are impaired in AF. To that end, Igarashi and colleagues used atrial gene transfer of Cx43 to reduce the incidence of AF by enhancing cell-to-cell coupling and conduction velocity in a porcine model [68]. In addition to electrical remodeling, seminal studies by Nattel and others have highlighted the importance of structural abnormalities in the pathophysiology of long-standing AF [69]. As a major component of structural remodeling, caspase 3, a key downstream enzyme in the signaling pathway leading to cardiomyocyte apoptosis, is upregulated in fibrillating atria [70]. By inactivating caspase 3 using a gene transfer approach, Trappe and colleagues were able to decrease the rate of apoptosis, improve intra-atrial conduction delays and inhibit the onset of persistent AF [71].

2.3. GENE THERAPY FOR BRADYARRHYTHMIAS IN HEART FAILURE

Defects in the specialized cardiac conduction system cause a variety of bradyarrhythmic disorders, including sick sinus syndrome and advanced heart block, both of which are associated with increased morbidity and mortality [72]. Hence, normalization of the rate and sequence of ventricular activation is an important therapeutic goal. Native cardiac pacemaking is generated in the SAN, a discrete structure located in the high right atrium. The wavefront then captures the surrounding atria and the AVN where it is delayed before transmission to the bundle of His, left and right bundle branches, and network of purkinje fibers. Subsequently, the depolarization wavefront undergoes transmural conduction from endocardium to epicardium.

Conduction system disease, which can lead to a range of bradyarrhythmic disorders in HF patients, is associated with increased morbidity and mortality [72]. As of yet, there are no successful pharmacological approaches for the treatment of bradyarrhythmic disorders. Instead, implantation of electronic pacemakers is required. Although highly effective, these

devices are subject to several limitations. For one, electronic pacemakers are not sensitive to autonomic regulation, and therefore, do not allow for heart rate modulation in response to changes in the sympatho-adrenergic state of the patient. Moreover, by imposing an abnormal activation sequence, pacemakers provoke significant ventricular remodeling [73]. In the long term, this may exacerbate the pathological substrate of the failing heart. Ventricular pacing from the LV myocardium may also introduce long intraventricular conduction delays which promote mechanical dyssynchrony, an effect that substantially worsens the outcome of patients with HF. Kass, Tomaselli, and others have identified the molecular and electrophysiological consequences of mechanical dyssynchrony in HF, and uncovered pathophysiologically-relevant heterogeneities that arise between the early and late-activated areas of the dyssynchronously-contracting failing LV [74, 75]. Finally, because of technical challenges, including a limited battery life, multiple invasive replacement procedures are necessary [76]. As expected, these surgical procedures carry a significant risk of inflammation [76]. Hence, development of novel treatment strategies for bradycardic disorders, such as biological pacemakers, is highly desirable.

2.3.1. Gene Therapy for Bradycardia: Development of Biological Pacemakers

—Major milestones have been achieved towards the development of novel biological pacemakers that can fully replace, or at least complement, existing electronic devices [77]. Our growing understanding of the molecular basis of cardiac pacemaking, including the revelation of a complex interplay between voltage and calcium clocks within the cardiac myocyte, has been instrumental in the identification of key targets that can be manipulated for the purpose creating viable biological pacemakers.

In proof-of-concept studies, Miake and colleagues [78] used viral gene transfer of an engineered dominant negative construct of Kir2.1 (Kir2.1AAA) to focally inhibit the inward rectifier potassium current, I_{K1}. This converted normally quiescent cells into ones that exhibited spontaneous depolarization and rhythmic activity. When injected into the LV, these constructs “captured” the myocardium resulting in premature ventricular beats that likely emanated from the site of injection [78]

In parallel studies, Rosen and colleagues developed HCN-based approaches for the creation of robust biological pacemakers [77, 79–82]. These constructs provided physiologically acceptable heart rates and elicited positive chronotropic responses to emotional arousal and stress. Indeed, the efficacy and translatability of these innovative approaches were documented in pre-clinical large animal models [77, 79–82]. For example, injection of HCN2 into the canine right atrium or left bundle branch improved the escape rhythm in response to vagal stimulation [82, 83]. Moreover, the same construct accelerated heart rate and reduced the need for device-mediated pacing following AVN ablation [84].

Other biological approaches focused on modifying the HCN channel structure to improve its pacemaking activity. Specifically, mutant or chimeric HCN constructs with altered activation kinetics (shifted to more positive potentials) were designed to enhance channel activity during diastole. While the combination of HCN2 and HCN1 in a chimeric construct resulted in VT [80], the HCN2 E324A point mutant resulted in a reasonable heart rate and a pronounced responsiveness to catecholaminergic stimulation. Gene therapy using this

mutant construct as opposed to the wildtype channel, however, did not provide additional benefits, possibly due to its relatively low expression levels [84]. The triple deletion (HCN1ΔΔΔ) construct yielded promising results in cardiomyocytes and *ex vivo* perfused guinea pig hearts. When injected into the left atrial appendage of SAN-ablated pigs, this construct created robust heart rates that were sensitive to catecholaminergic stimulation [85].

Because I_f is highly regulated by adrenergic mechanisms, similar results have been achieved by stimulating cAMP production directly using AC gene transfer [86]. Injection of the Ca^{2+}-stimulated AC1 isoform of this enzyme increased basal heart rate and reduced the need for electronic pacing [87]. Boink and colleagues examined the efficacy of a combined HCN2 and AC1 gene transfer approach. While this strategy further improved the autonomic responsiveness of the biological pacemaker, it resulted in excessively fast heart rates [87].

Other combinatorial strategies for creating robust biological pacemakers were also tested. One such approach involved the combination of HCN2 with constructs designed to downregulate I_{K1} or upregulate I_{Na}. Indeed, co-expression of HCN2 and the dominant negative Kir2.1AAA construct in the AVN junction region of AVN-ablated pigs achieved reasonable results with a substantial (30%) increase in heart rate and a 5-fold reduction in the percentage of electronically-mediated beats [88]. Of note, gene transfer in this study was performed by percutaneous gene delivery using the femoral vein [88], a procedure that is highly amenable for translation to humans. The alternative combinatorial approach (HCN2 and SkM1) was skillfully designed to promote successful pacemaking by increasing the driving force of the biological pacemaker through enhancement of I_{Na} [89]. In comparison to HCN2 overexpression alone, transfer of both gene constructs (HCN2 and SkM1) into the left bundle branch of AVN-ablated dogs resulted in a more robust heart rate. Remarkably, this strategy completely eliminated the dependency of animals on electronic pacemakers and improved their circadian rhythm and adrenergic response [90].

More recently, alternative approaches for the generation of biological pacemakers have been advanced. Specifically, the use of gene transfer to reprogram quiescent myocardial cells into pacemakers by switching on their pluripotent state was tested. Leading molecular candidates for achieving this switch are the T-box transcription factors TBX3 and TBX18, both of which regulate the cardiac conduction system during early development [91]. When expressed in murine cardiomyocytes, TBX3 successfully induced their differentiation into pacemaker cells *in vitro* [92]. Even more robust effects were shown for TBX18 which unleashed ectopic pacemaker activity both *in vitro* and *in vivo* in a guinea pig model [93]. This minimally invasive somatic reprogramming approach was subsequently implemented in a large animal model of heart block produced by AVN ablation. Hu and colleagues [94] demonstrated pacemaker-like activity at the site of injection. During follow up for 14 days, TBX18-treated animals exhibited reasonable heart rates that were modestly faster than those of their GFP-expressing counterparts. Furthermore, TBX18-treated animals exhibited a minimal need for electronic pacing and an enhanced autonomic responsiveness [94]. An attractive feature of targeting transcription factors for creating “inducible pacemaker cells” is their presumed ability to produce long term effects even after the expression of the target gene (in this case TBX18) has vanished. This unique property lends itself well for an adenoviral-mediated gene delivery approach that offers relatively transient expression of

TBX18. Nonetheless, chronic monitoring in clinically relevant models of sick sinus syndrome or heart block are warranted to assess the long-term safety of this somatic reprogramming approach. Key issues need to be carefully considered when adopting a TBX-based strategy for biological pacemaking [95]. These include a systematic assessment of the long-term resiliency of the so-called inducible pacemakers against cell death in the wake of chronically elevated cAMP levels. Moreover, the proliferative effect of TBX18 on non-myocytes (namely fibroblasts) needs to be examined. Excessive fibroblast proliferation at the site of TBX18 injection may certainly exert deleterious effects through myocyte-fibroblast interactions (both electrical and biochemical). Finally, the potential for these transcription factors to promote neoplasm formation needs to be addressed.

3. EXPERT OPINION: GENE THERAPY FOR ARRHYTHMIAS IN HF

Existing pharmacological and device therapies for patients with HF are far from optimal. Therefore, the search for novel mechanism-based therapeutic options for these patients is critical. Although cardiac gene therapy may be a natural candidate for treating inherited arrhythmias, its potential for a much broader applicability has become increasingly apparent. Indeed, the evolution of efficient, cardiac-specific gene transfer technologies, coupled with the identification of key molecular deficits, have placed HF-related arrhythmias well within reach of gene-based therapies [7, 96]. A major advantage of gene therapy for the treatment of arrhythmias is the ability to selectively target specific cell types (such as myocytes) in the heart while sparing other cell populations. This allows one to achieve the desired goal while avoiding confounding effects (for example, hypotension in the case of pharmacological beta blockers).

The successful use of cardiac gene therapy for arrhythmias entails a careful selection of the specific vector, serotype, promoter, and delivery technique. For a detailed review of methods used to achieve stable cardiac-specific gene transfer, the reader is referred to excellent reviews on the subject [97, 98]. Major considerations include whether transient or chronic gene expression is needed, which specific cell type requires modification, and whether gene transfer is to be applied locally or globally. Since targeted gene delivery strategies allow one to limit the therapeutic effect to exactly the area that requires modification (such as the epicardial border zone of an infarct or discrete structures in the cardiac conduction system), one is able to avoid unintended proarrhythmic effects in other regions.

Key milestones in our effort to develop novel gene therapy approaches for supraventricular tachy and brady-arrhythmias have been achieved. Indeed, the successful creation of biological pacemakers, beta-blockers and calcium channel blockers are major breakthroughs that will likely impact patient care in the not-so-distant future. Despite these impressive achievements, substantial hurdles still exist for treating and/or preventing VT in HF using gene-based approaches. These include our incomplete understanding of the molecular basis of excitability and the multiplicity of ion channel effectors that give rise to VT in the failing heart. Indeed, heterogeneous ion channel expression [99, 100], the delicate balance between numerous voltage- and time-dependent ion conductances, and the complex cross-talk between voltage and intracellular calcium present major challenges in combatting HF-related VT. It is noteworthy that the most effective anti-arrhythmic drugs at our disposal

(such as amiodarone) are poorly selective agents that elicit non-specific effects on multiple ion channels. In fact, selective anti-arrhythmic drugs often promote rather than prevent VT. This begs the question of whether a “silver bullet” approach is beneficial or counterproductive in situations where widespread ion channel remodeling has already developed. We believe that the ultimate success of a given gene therapy approach will depend on a clear understanding of the molecular basis of the specific arrhythmia. With regards to HF-related VT, a detailed knowledge of the dynamic nature and time-course of ion channel remodeling that promotes the formation of arrhythmogenic triggers and substrate is required. We envision that gene therapy may serve as a preventive rather than ameliorative measure for complex arrhythmias that depend on a host of electrical, mechanical, structural, and metabolic alterations. In those situations, we believe that early targeting of disease promoting pathways (rather than end effectors of excitability) may be advantageous as this strategy may hinder overall disease progression. Moreover, gene therapy may be particularly useful in suppressing arrhythmogenic triggers if reversal of the substrate is not realistic. The ability of gene therapy to convert arrhythmias after significant electrical, structural, and metabolic abnormalities have developed will require detailed investigation in the future.

Another major issue that warrants exhaustive investigation is the potential for gene therapy approaches to induce electrophysiological toxicities. The “safety margin” of a given gene therapy approach should be determined by assessing its dose-response profile. Is it possible to overexpress a given ion channel gene to such artificial levels that ultimately prove to be counterproductive? In an effort to normalize repolarization of the failing heart, are we at risk of converting what is in essence an acquired form of the Long QT Syndrome into a short QT phenotype, which is just as, if not more, proarrhythmic? Lessons learned from transgenic mouse models indicate the potential for early lethality by excessive overexpression and/or silencing of certain genes. Even when an appropriate dose is used, considerable effort is required to avoid introducing regional and transmural gradients in the expression levels of some ion channels which are known to promote arrhythmogenesis when they are heterogeneously expressed.Finally, gene therapy applications in HF require long term gene modification (with the exception of the transcription factors discussed above). While existing AAV-based approaches are effective, they still require significant optimization. A major issue is the presence of antecedent neutralizing antibodies against AAV vectors in a significant proportion (up to 50% for AAV1) of patients. This limitation, however, appears to be surmountable as engineered hybrid vectors and chimera are being developed and tested. These novel vectors are designed to elude recognition by neutralizing antibodies while at the same time exhibiting superior cardiac tropism.

In summary, highly promising approaches for restoring heart rate and rhythm by targeting myocardial conduction, repolarization, calcium cycling, and adrenergic signaling have been elegantly conducted in small and large animal models (see Tables 1–4). These important milestones, coupled with major improvements in vector technology and efficient gene delivery methods, foreshadow a bright future for cardiac gene therapy in the treatment and prevention of arrhythmias in heart failure.

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ARTICLE HIGHLIGHTS BOX

- Heart failure is a major public health epidemic and a leading cause of atrial and ventricular arrhythmias.
- Existing pharmacological and device strategies for the treatment of arrhythmic disorders are suboptimal.
- Major advances in our basic understanding of arrhythmia mechanisms and in gene transfer techniques have placed arrhythmias well within reach of gene-based therapies
- Gene therapy approaches have been developed to combat VT by restoring repolarization, conduction, and calcium cycling properties in HF.
- Biological pacemakers and beta-blockers have been designed to manage bradyarrhythmias and supraventricular tachyarrhythmias, respectively.

Table 1

Gene therapies for ventricular tachycardia

	Vector	*Delivery technique*	*Genetic information*	*Species*	*Target tissue*	*Outcomes*
Murata et al., 2004	adenovirus	injection into LV cavity	mutant ras-related G-protein Gem W296G	guinea pig	VM	↓I_{CaL} in cardiomyocytes, ↓QT in *vivo*
Cingolani et al., 2007	lentivirus	injection into LV cavity	hairpin RNA for β_2 LTCC	rat	VM	↓ I_{CaL} in cardiomyocytes
Lebeche et al., 2004	adenovirus	injection into aortic root	human Kv4.3	rat	VM	↑ I_{to} & ↓ APD in cardiomyocytes
Mazhari et al., 2002	adenovirus	injection into LV cavity	human MiRP2	guinea pig	VM	↑ I_{Ks} & ↓ APD in cardiomyocytes, ↓ QT *in vivo*
Greener et al., 2012	adenovirus	antero & retrograde coronary injection	rat Cx43	swine	VM (BZ MI)	↓ EG fractionation, ↑ CV, ↓ VT
Lau et al., 2009	adenovirus	intramyocardial injection	SkM1	dog	VM (BZ MI)	↓ EG fractionation, ↓ VT
Prestia et al., 2011	adenovirus	intramyocardial injection	murine Cx32	mouse	VM	↑ infarct size, ↔ VT
Anuykhovsky et al., 2011	adenovirus	intramyocardial injection	rat SkM1 and/or murine Cx32	mouse	VM	↑ CV (SkM1), ↓ VT during IR (SkM1, Cx32)
Boink et al., 2012	adenovirus	intramyocardial injection	rat SkM1 and/or murine Cx32	dog	VM (BZ MI)	↓ EG fractionation, ↓ QRS duration; ↓ VT (SkM1), ↔ VT (Cx32, SkM1+Cx32), ↑ infarct size (Cx32)
Sasano et al., 2006, 2009	adenovirus	antero & retrograde coronary injection	mutant HERG G628S	swine	VM (BZ MI)	↑APD, ↑ ERP, ↓ VT
Cutler et al., 2009	adenovirus	injection into aortic root	SERCA2a	guinea pig	VM	↓ APD alterans *in vitro* & *ex vivo*, ↓ VT *ex vivo*
Cutler et al., 2013	AAV9	injection into aortic root	SERCA2a	guinea pig	VM	↓ APD alterans & ↓ VT *ex vivo*
Lyon et al., 2011	adenovirus AAV9	intramyocardial (adenovirus) or tail vein (AAV9)	SERCA2a (human for AAV9)	rat	VM	↓ VT *ex vivo*; ↓ spontaneous & isoproternol triggered VT *in vivo*
del Monte et al., 2004	adenovirus	intramyocardial injection	SERCA2a	rat	VM	↓ VT after I/R
Prunier et al., 2008	adenovirus	anterograde coronary injection	SERCA2a	swine	VM	↓ VT after I/R

The origin of the exogenously delivered genetic material is indicated if specified in the study. Injection into the aortic root or the LV cavity was performed during transient cross clamping of the great vessels. Intramyocardial injection was performed after thoracotomy. AAV9, adeno-associated virus serotype 9; APD, action potential duration; BZ, border zone; CV, conduction velocity; ECG, electrocardiogram; EG, electrogram; ERP, effective refractory period; HF, heart failure; I/R, ischemia reperfusion; LTCC, L-type calcium channel; LV, left ventricle; MI, myocardial infarction; VM, ventricular myocardium; VT, ventricular tachycardia.

Table 2

Gene therapies for supraventricular tachyarrhythmias

	Vector	*Delivery technique*	*Genetic information*	*Species*	*Target*	*Outcomes*
Donahue et al., 2000	adenovirus	anterograde coronary injection	rat $G\alpha_{i2}$	swine	AVN	↑ ERP in AVN, ↓ HR during AF
Bauer et al., 2004	adenovirus	anterograde coronary injection	rat $G\alpha_{i2}$ and mutant Cre-Lox recombinant $cG\alpha_{i2}$	swine	AVN	↓ HR in persistent AF
Murata et al., 2004	adenovirus	anterograde coronary injection	mutant ras-related small G-protein Gem W296G	swine	AVN	↓ conduction in AVN, ↓ HR during AF
Lungenbiel et al., 2012a	adenovirus	anterograde coronary injection	mutant siRNA-$G\alpha_s$	swine	AVN	↓ conduction in AVN, ↓ HR in persistent AF
Lungenbiel et al., 2012b	adenovirus	intramyocardial injection after TT	mutant siRNA-$G\alpha_s$	swine	SAN	↓ HR during SR
Amit et al., 2010	adenovirus	epicardial gene painting	mutant human ERG G628S	swine	atrium	↑ atrial APD, ↓ risk of AF
Soucek et al., 2012	adenovirus	atrial local injection + electroporation	mutant canine ERG G628S	swine	atrium	↑ atrial ERP, ↓ onset of AF
Igarashi et al., 2012	adenovirus	epicardial gene painting	human Cx40 or rat Cx43	swine	atrium	↑ intra-atrial conduction during AF *ex vivo* & *in vivo*, ↓ risk of AF
Trappe et al., 2013	adenovirus	atrial local injection + electroporation	mutant siRNA-Cas3	swine	atrium	↓ apoptosis, ↓ intra-atrial conduction heterogeneity, ↓ onset of AF

The origin of the exogenously delivered genetic material is indicated if specified in the study. Injection into the aortic root or the LV cavity was performed during transient cross clamping of the great vessels. Intramyocardial injection was performed after thoracotomy. AF, atrial fibrillation; APD, action potential duration; AVN, atrioventricular node; ERP, effective refractory period; HR, heart rate; SAN, sinoatrial node; SR, sinus rhythm; TT, thoracotomy.

Table 3

Gene therapies for bradyarrhythmias

	Vector	*Delivery technique*	*Genetic information*	*Species*	*Target*	*Outcomes*
Miake et al., 2002	adenovirus	injection into LV cavity + transient cross clamping of great vessels	mutant Kir2.1AAA	guinea pig	VM	spontaneous pacemaker activity *in vitro*, ↑ ventricular rate on ECG
Qu et al., 2003	adenovirus	intramyocardial injection after TT	murine HCN2	dog	LA appendage	↑ I_f, ↑ escape rhythm during VS
Plotnikov et al., 2004	adenovirus	percutaneous intramyocardial injection	murine HCN2	dog	LBB	↑ I_f, ↑ escape rhythm during VS
Bucchi et al., 2006	adenovirus	percutaneous intramyocardial injection	murine HCN2 or HCN2 E324A	dog	LBB	↑ HR, ↓ PMI (HCN2E324A = HCN2), ↑ AR (HCN2E324A > HCN2)
Tse et al., 2006	adenovirus	intramyocardial injection after TT	mutant murine HCN1ΔΔΔ	guinea pig; swine	VM; LA appendage	spontaneous PM activity *in vitro & ex vivo*, ↑ HR & ↓ PMI in SSS
Plotnikov et al., 2008	adenovirus	percutaneous intramyocardial injection	murine HCN2 or chimeric HCN212	dog	LBB	VT (with HCN212)
Ruhparwar et al., 2010	adenovirus	intramyocardial injection after TT	murine AC6	swine	VM	creation of escape rhythm in cAVB
Boink et al., 2012	adenovirus	percutaneous intramyocardial injection	HCN2 (murine) and/or AC1	dog	LBB	cAVB: ↓ PMI (HCN2+AC1, AC1 > HCN2), ↑ HR (HCN2+AC1 > AC1, HCN2), excessive HR with HCN2+AC1
Cingaloni et al., 2012	adenovirus	intramyocardial injection after TT percutaneous via venous catheter	murine HCN2 or/and Kir2.1AAA	swine	VM; AV junction	↑ HR & ↓ PMI in cAVB
Boink et al., 2013	adenovirus	percutaneous intramyocardial after TT	murine HCN2 or/and rat SkM1	dog	LBB or VM	cAVB: no PMI in HCN2+SkM1, ↑ HR (HCN2+SkM1 > HCN2 or SkM1), ↑ AR & ↑ circadian rhythm modulation (HCN2+SkM1 > HCN2 or SkM1 alone)
Hu et al., 2014	adenovirus	percutaneous intramyocardial injection via venous catheter	human TBX18	swine	upper posteroseptal RV	In cAVB: creation of PM cells, ↑ HR, ↓ PMI, ↑ autonomic response

Study characteristics of gene therapy approaches for bradyarrhythmias. The origin of the exogensouly delivered genetic material is indicated if specified in the study. AR, adrenergic response; AV, atrioventricular; HR, heart rate; cAVB, complete atrioventricular block; ECG, electrocardiogram; LA, left atrium; LBB, left bundle branch; LV, left ventricle; PM, pacemaker; PMI, electronic pacemaker intervention; RV, right ventricle; VM, ventricular myocardium; SSS, sick sinus syndrome; TT, thoracotomy; VS, vagal stimulation; VT, ventricular tachycardia.

Table 4

Stage of development of selected gene therapy approaches

Therapeutic Goal	***Principle***	***Vehicle & Delivery Method***	***Development & Translation***	***Reference***
Block depolarizing Ca channels	↓ I_{Ca} in VM	Adenovirus + lentivirus, injection into LV cavity	*in vitro* & *in vivo* in rodents	*Murata et al., 2004; Cingolani et al., 2007*
Increase repolarizing K channels (I_{to})	↑I_{to} in VM	Adenovirus, injection into aortic root	*in vitro* in rodents	*Lebeche et al., 2004*
Improve conduction	↑ intercellular coupling in MI BZ in VM	Adenovirus, anterograde & retrograde coronary injection	*in vivo* in large animals	*Greener et al., 2012*
Improve conduction	↑ I_{Na} in MI BZ in VM	Adenovirus, intramyocardial injection after TT	*in vivo* in large animals	*Lau et al., 2009; Boink et al., 2012a*
Prolong local refractoriness	↓ I_{Kr} in MI BZ in VM	Adenovirus, anterograde & retrograde coronary injection	*in vivo* in large animals	*Sasano et al., 2006,2009*
Accelerate Ca-cycling in HF	↑ SR Ca reuptake in VM	Adenovirus, intramyocardial or via aortic root AAV9, tail vein injection	*ex vivo* and *in vivo* in rodents	*Lyon et al., 2011; Cutler et al., 2013*
Accelerate Ca-cycling in MI	↑ SR Ca reuptake in VM	Adenovirus, anterograde coronary injection	*in vivo* in large animals	*Prunier et al.,2008*
Biological beta blocker for rate control	↓ adrenergic response in AVN	Adenovirus, anterograde coronary injection	*in vivo* in large animals	*Donahue et al., 2000; Bauer et al.,2004; Lungenbiel et al., 2012a*
Biological Ca blocker for rate control	↓ I_{Ca} in AVN	Adenovirus, anterograde coronary injection	*in vivo* in large animals	*Murata et al., 2004*
Achieve rate control	↓ adrenergic response in SAN	Adenovirus, intramyocardial injection after TT	*in vivo* in large animals	*Lungenbiel et al., 2012b*
Increase refractoriness	↓ I_{Kr} in atrium	Adenovirus, epicardial gene painting or local injection & electroporation	*in vivo* in large animals	*Amit et al., 2010; Soucek et al., 2012*
Improve conduction	↑ intercellular coupling in atrium	Adenovirus, epicardial gene painting	*ex vivo & in vivo* in large animals	*Igarashi et al., 2012*
Improve structural remodeling	↓ apoptosis in atrium	Adenovirus, local injection + electroporation	*in vivo* in large animals	*Trappe et al., 2013*
Create biological pacemaker	↑ I_f and/or ↑ adrenergic response in LBB	Adenovirus, percutaneous intramyocardial injection	*in vivo* in large animals	*Boink et al., 2012b*
Create biological pacemaker	↑ I_f and ↓ I_K in LBB	Adenovirus, percutaneous intramyocardial	*in vivo* in large animals	*Cingolani et al., 2012*
Create biological pacemaker	↑ I_f and ↑ I_{Na} in LBB	Adenovirus, percutaneous intramyocardial	*in vivo* in large animals	*Boink et al., 2013*
Create biological pacemaker	somatic reprogramming by ↑TBX18	Adenovirus, percutaneous intramyocardial	*in vivo* in large animals	*Hu et al., 2014*

AAV9, adeno-associated virus serotype 9; AF, atrial fibrillation; AVN, atrioventricular node; BZ, border zone; HF, heart failure; LBB, left bundle branch; LV, left ventricle; MI, myocardial infarction; SAN, sinoatrial node; SR, sarcoplasmatic reticulum; TT, thoracotomy; VM, ventricular myocardium; VT, ventricular tachycardia.