Molecular therapies for cardiac arrhythmias
Boink, G.J.J.

Citation for published version (APA):
Boink, G. J. J. (2013). Molecular therapies for cardiac arrhythmias

General rights
It is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), other than for strictly personal, individual use, unless the work is under an open content license (like Creative Commons).

Disclaimer/Complaints regulations
If you believe that digital publication of certain material infringes any of your rights or (privacy) interests, please let the Library know, stating your reasons. In case of a legitimate complaint, the Library will make the material inaccessible and/or remove it from the website. Please Ask the Library: http://uba.uva.nl/en/contact, or a letter to: Library of the University of Amsterdam, Secretariat, Singel 425, 1012 WP Amsterdam, The Netherlands. You will be contacted as soon as possible.
EMERGING THERAPIES TARGETING MYOCARDIAL INFARCTION-RELATED ARRHYTHMIAS

Gerard JJ Boink

Heart Center, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands, and Interuniversity Cardiology Institute of the Netherlands, Utrecht, The Netherlands.
**ABSTRACT**

Cardiac arrest is the number one cause of death in the developed world. Ventricular arrhythmias relating to ischemia and myocardial infarction are a major contributor and therefore require aggressive treatment. Drugs, devices and radiofrequency catheter ablation have made inroads, but suffer from important limitations ranging from incomplete success to significant toxicities and major side effects. These limitations derive from the nature of the intervention: drugs target the entire heart rather than a specific substrate for arrhythmias, devices terminate rapid rhythms but do not impact on the underlying disease, and ablation is appropriately targeted but results in tissue damage. In an effort to resolve this suboptimal state of affairs, gene and cell therapies are being explored to provide a targeted, non-destructive and potentially regenerative approach to ventricular arrhythmias resulting from myocardial infarction. Although these approaches are still in early stages of development, they are expected to revolutionize the way in which we treat arrhythmias.
INTRODUCTION

Heart disease is the leading cause of death in the developed world killing nearly 600,000 individuals in 2010 in the U.S. alone. Ventricular arrhythmias associated with ischemia and myocardial infarction are a major contributor: the predominant underlying electrophysiological mechanism is reentry. Early in the 20th century, Mayer laid the foundation for our understanding of reentry. He induced sustained rhythmic muscle activation circling a ring cut from Scyphomedusae subumbrella tissue. Mines expanded on these experiments using vertebrate hearts and importantly suggested a link between reentry and the occurrence of clinical arrhythmias. In the process, he predicted the mechanism of Wolff-Parkinson-White (WPW) Syndrome and also described the interventions that would have to be performed to diagnose reentry as the mechanism.

These early insights noted that reentry may be prevented by strategies that generate bidirectional conduction block by either blocking conduction or prolonging refractoriness, or by restoring normal, fast conduction in depressed areas. It took, however, until the 1970s before mechanistic insight implicating reentry could be definitively confirmed. In patients with the WPW Syndrome, premature ventricular stimulation and epicardial mapping studies had suggested an accessory atrio-ventricular pathway to be responsible for the circus movement tachycardia. Yet, important confirmation of this hypothesis came from surgical studies in which such a pathway could be identified using epicardial mapping followed by interruption of the pathway via incision. Successful cases showed disappearance of preexcitation, as evidenced by loss of the delta wave on the ECG, in conjunction with removal of the tachycardia.

In the 1970s as well, more drugs that slow conduction and drugs that prolong refractoriness were becoming available, and experimental studies indicated significant potential for these interventions in terminating reentrant pathways. Yet, during those days, the application of antiarrhythmic drugs was rather empirical. Following the Lown classification, the hypothesis was that suppressing premature ventricular depolarizations after myocardial infarction would suppress lethal arrhythmias and thereby mortality. This initially led to the Cardiac Arrhythmia Pilot Study (CAPS) which showed that Na+ channel blockers encainide and flecainide met the pre-specified efficacy criteria of more than 75% reduction in the number of ectopic beats.

Outcomes of the CAPS trial were interpreted as needing confirmation in larger scale follow-up studies leading to the Cardiac Arrhythmia Suppression Trial (CAST). This study investigated both arrhythmia suppression and mortality in patients post myocardial infarction. Yet, the study was terminated prior to completion as interim reports indicated that encainide, flecainide and moricizine were associated with a higher mortality than placebo, particularly related to sudden cardiac death. While this was a huge setback to the field, CAST focused increased attention on the mechanism of drug action. Since class I antiarrhythmics had failed, it was proposed that a different class of antiarrhythmics (i.e., class III) might provide the required specificity needed
to prevent lethal post myocardial infarction arrhythmias. To this end, the Survival With Oral D-sotalol (SWORD) trial was initiated. This study investigated a specific blocker of repolarizing current, as the d-enantiomer of sotalol lacks \( \beta \)-adrenergic blocking actions of the d,l-racemate. Yet, again mortality was higher than with placebo.\(^{14}\) While excess proarrhythmia was thought to be a major cause of death in SWORD, specific arrhythmias, particularly **torsade de pointes** ventricular tachycardia, could not be linked to the study’s fatalities.\(^{15}\) In the meantime, the Conventional versus Amiodarone (a class I, II, III and IV blocker) Drug Evaluation (CASCADE) study did show reduced total cardiac mortality in post myocardial infarction patients. Yet, mortality rates remained high and amiodarone use was associated with significant thyroid dysfunction and pulmonary toxicity.\(^{16}\)

To date, antiarrhythmic drug therapy has still not reached the success that was hoped for. Radiofrequency (RF) ablation provides a more targeted intervention, but efficacy has been incomplete and its precision is not sufficient to avoid major myocardial damage. Furthermore, the implantation of cardioverter/defibrillators saves many lives, yet their painful shocks importantly call for therapies that maximally reduce their need or intervention rate.\(^{17}\) With the arrival of gene and cell therapy, new opportunities have surfaced for the development of such therapies. The antiarrhythmic strategy of speeding conduction, that had been difficult pharmacologically, is now within reach. Gene therapy to improve conduction indeed demonstrated encouraging outcomes with regards to the prevention of ventricular tachycardia/ ventricular fibrillation (VT/VF).\(^{18-21}\) However, it remains uncertain to what extent the prevention of these arrhythmias is critically linked to: 1) the gene intervention used, 2) the degree of conduction normalization obtained, and 3) the delivery vehicle involved. In part IV of this thesis, we therefore focused on answering these questions.

In chapter 11, we compare several gene interventions aimed at speeding conduction in a canine model of subacute myocardial infarction.\(^{22}\) Here, high extracellular potassium concentrations and acidification lead to depolarization induced Na\(^+\)-channel inactivation and closure of Cx43 gap junctions; both changes importantly contribute to slow conduction. The following selected gene interventions were specifically designed to withstand these ischemic conditions: 1) the depolarization resistant skeletal muscle Na-channel (SkM1), 2) the acidification resistant connexin (Cx32), and 3) the combination of both SkM1 and Cx32. Seven days after adenoviral construct implantation and creation of myocardial infarcts, we proceeded with \textit{in situ} electrophysiological testing. We found that all three interventions normalized conduction, as evidenced by significantly shorter QRS durations, narrower local electrograms, and faster \textit{in vitro} conduction velocities. Furthermore, a subanalysis of QRS duration during premature electrical stimulation (PES) from the epicardial borderzone (EBZ) showed fastest ventricular activation in the SkM1/Cx32 group. However, only the SkM1 intervention significantly reduced the incidence of inducible VT/VF, thus indicating the criticality of the genetic intervention involved and the level of conduction restoration obtained.
Given the more favorable outcomes for SkM1 in restoring rapid impulse propagation and preventing VT/VF, chapters 12 and 13 focus on testing an alternative delivery vehicle for this approach. We speculated that a cellular delivery platform could positively impact on damaged myocardium and therefore might provide an attractive alternative to the viral approach. To this end, we started testing the delivery of SkM1 via gene-modified human embryonic kidney (HEK) cells. Because these cells endogenously express the cardiac connexin (Cx43) and are easily manipulated in the laboratory, they provide an attractive initial screening tool for cellular delivery of ion channels. Similar to previous work with viral delivery, we also asked if delivery of SkM1 would be superior to delivery of the Nav1.5 cardiac Na-channel. Using patch-clamp and optical mapping, we confirmed feasibility and superiority for cellular delivery of SkM1 in restoring the action potential (AP) upstroke and the speed of conduction in depolarized tissue. These outcomes encouraged us to move the cellular SkM1 approach forward into intact canine testing.

In chapter 13 we use the mesenchymal stem cell platform to deliver SkM1 into the EBZ of infarcted canine heart. We studied 3 groups of dogs: sham, canine mesenchymal stem cells (cMSC) and cMSC/SkM1 and found in vivo EBZ electrograms to be broad and fragmented in sham, narrower but still fragmented in cMSC, and narrow and unfragmented in cMSC/SkM1. Furthermore, during PES of EBZ, QRS duration in cMSC/SkM1 was shorter than in cMSC and sham. However, despite the potentially therapeutic actions of cMSC/SkM1, no protection against arrhythmias was obtained, as PES-induced VT/VF was equivalent in all groups. When comparing the outcomes of cellular and viral delivery of SkM1, we concluded that the viral delivery method is superior for obtaining the SkM1-based antiarrhythmic effect.

The present chapter discusses the various approaches aimed at speeding conduction and tries to answer why they sometimes succeed and sometimes fail in the prevention of VT/VF. Subsequently, we will place the successful strategies into perspective with other emerging therapies targeting myocardial infarction-related arrhythmias and recapitulate the steps to be taken in moving these therapies forward towards clinical application.

GENE THERAPY TO IMPROVE CONDUCTION AND PREVENT REENTRY-BASED VT/VF

Several studies have shown favorable outcomes using gene therapy approaches to speed conduction and prevent VT/VF. Initial proof-of-concept for sodium channel overexpression came from Lau and colleagues who used adenoviral gene transfer to overexpress SkM1 into the EBZ of infarcted canine hearts. In this setting, they found significantly reduced arrhythmia inducibility, normalized local electrogram duration in the intact heart, and increased phase 0 upstroke velocity in APs of depolarized myocardium excised from the heart. In a subsequent epicardial mapping study, the investigators showed that SkM1 overexpression significantly increased longitudinal conduction in the EBZ. Furthermore, SkM1 gene transfer also reduced the incidence
of spontaneous arrhythmia in a murine model of ischemia-reperfusion. Reentry is considered to be the major mechanism involved in arrhythmogenesis in both models. Hence, SkM1 overexpression improves upstroke velocity in depolarized tissue, speeds longitudinal conduction, and prevents reentry-based arrhythmias in settings of myocardial infarction and ischemia-reperfusion.

The other major approach to normalizing conduction, centers around improving gap junction function. Here, Cx32 overexpression reduced arrhythmia incidence as effectively as SkM1 in mice following ischemia/reperfusion. Similarly, Cx43 gene transfer was tested in porcine hearts manifesting inducible monomorphic VT. In this model, infarcts were created via temporary LAD occlusion. Four weeks later, animals with inducible sustained monomorphic VT were treated with adenoviral vector infusion into the infarcted area. Cx43 gene transfer reduced electrogram fractionation, improved conduction velocity and decreased arrhythmia inducibility from 100% to 40%. A concern with both connexin gene transfer strategies employed is that, in settings of acute myocardial infarction, they can increase the spread of mediators of injury from cell to cell, thereby increasing the size of the damaged area. This concern was supported by murine and canine studies in which infarct size indeed was larger after Cx32 gene transfer. Although Cx43 channels close upon ischemia induced acidification (potentially reducing the risk for larger infarcts), this process is incomplete. Concerns with regards to connexin overexpression in settings of ischemic heart disease therefore apply to all the isoforms tested.

Another approach to speed conduction and prevent reentry-based arrhythmias may stem from interference with the micro RNA, miR-1. MiR-1 levels are elevated in clinical and experimental settings of myocardial infarction, resulting in reduced expression of Cx43 and Kir2.1. Their outcomes are depolarized cell membranes and slowed conduction. Based on this knowledge, Yang and colleagues tested antagonim inhibition of miR-1 in myocardially infarcted rats in which they found more hyperpolarized membrane potentials, accelerated conduction and suppressed ventricular arrhythmias. In a different study investigating micro RNA-related arrhythmogenesis, miR-1 overexpression increased Ca^{2+}-cycling participating in isoproterenol-induced arrhythmias in vitro. Although both studies indicate a role for suppressing miR-1 as an antiarrhythmic approach, the large number of processes...
the same time infarct size was increased (as shown) which generated a substrate for monomorphic tachycardia possibly circling the larger infarct. In Ad-SkM1/Cx32 and cMSC/SkM1 treated animals, conduction was improved and infarct size remained unchanged, yet no protection against reentrant arrhythmias was generated. Possibly, the Ad-SkM1/Cx32 and cMSC/SkM1 interventions improved conduction in reentrant circuits that otherwise would have remained silent (represented by the differently positioned circuits), thereby maintaining a substrate for polymorphic arrhythmias. Note that the reentrant circuit is slightly larger than in control and SkM1 such that, despite the improved conduction, reentry can still persist. The tracings shown are from canines treated with Ad-GFP, Ad-SkM1, Ad-Cx32 and Ad-SkM1/Cx32, respectively, in which arrhythmias (except for Ad-SkM1) were induced by premature stimulation. See text for further details. EBZ: epicardial borderzone.
regulated by miR-1 may add to the complexity of such a strategy. For example, reductions in miR-1 have also been found in association with hypertrophic remodeling during heart failure.\cite{31,32}

**NORMALIZING CONDUCTION: WHY SOME STRATEGIES PREVENT VT/VF AND OTHERS DON’T**

The finding that strategies which provide comparable efficiency in restoring impulse propagation impact differently on arrhythmia inducibility is intriguing. Understanding the settings in which proposed therapies work or fail may help in targeting therapies to selected patients, thereby improving the overall safety and efficacy. So what have we learned from strategies that speed conduction but fail to prevent arrhythmias?

I will start by comparing the efficacy of various strategies that speed conduction. For this comparison I will focus on the data that are available from mice and dog studies as presented in Table 1. In *in vitro* studies, conduction velocities were recorded from isolated murine right ventricular preparations treated with SkM1 and Cx32 overexpression. At baseline, the conduction velocity is faster in SkM1 vs control, whereas Cx32 does not differ from control. This corresponds to the baseline murine QRS duration which is also shorter in SkM1 vs control and is likely caused by the relatively large abundance of connexins in healthy tissue. Hence, increasing the number of gap-junctions at baseline does not affect conduction. Sodium channels, on the other hand, appear to be a rate limiting factor in normal conduction. Despite these differences at baseline, SkM1 and Cx32 were remarkably similar in restoring impulse propagation during combined application of high potassium (generating membrane depolarization-induced inactivation of cardiac Na+-channels) and low pH (inducing closure of gap junctions).\cite{18} Similarly, in canine subacute myocardial infarcts, QRS duration (during sinus rhythm and PES) and electrogram duration in SkM1 and Cx32 were all significantly shorter than in controls. In canines, we also studied combined SkM1/Cx32 overexpression. Here, outcomes with regard to baseline QRS duration and local electrogram duration were comparable to the single gene interventions. What did differ was activation of myocardium during PES. Here, QRS duration was shorter than both control and the single gene interventions of SkM1 and Cx32.\cite{22}

A final intervention studied in canine was cMSC/SkM1, which was compared to the injection of unmodified cMSCs and uninjected sham animals. The finding that cMSC and cMSC/SkM1 generated a relatively comparable shortening of QRS duration in infarcted canine myocardium was unexpected. Yet, differences between these two interventions became apparent when we compared the duration of local electrograms and found outcomes in cMSC/SkM1 to be superior as compared to cMSC. When comparing QRS and local electrogram durations between stem cell and viral delivery of SkM1, outcomes appeared comparable.\cite{26}

Another aspect to consider in comparing the above discussed studies is the area at risk or the area of tissue damage. This distinction stems from the model being applied.
Table 1. Summary data of the various gene and cell therapeutic approaches to speed conduction and prevent arrhythmias in ischemia/reperfusion mice and canine with subacute myocardial infarction.

<table>
<thead>
<tr>
<th>Outcomes</th>
<th>Ad-Empty, Ad-GFP</th>
<th>Ad-SkM1</th>
<th>Ad-Cx32</th>
<th>Ad-SkM1/Cx32</th>
<th>Sham</th>
<th>cMSC</th>
<th>cMSC/SkM1</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Subacute myocardial infarction (mice)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PES-induced VT (%)</td>
<td>55</td>
<td>50</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Infarct size (%)</td>
<td>23±</td>
<td>44±5*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Ischemia/reperfusion (I/R; mice)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>QRS (ms; before I/R)</td>
<td>11±0.3</td>
<td>9±0.3*</td>
<td>12±0.4</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>In vitro CV (ms; baseline)</td>
<td>33±2</td>
<td>43±3</td>
<td>32±2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>In vitro CV (ms; high K+ low pH)</td>
<td>17±2^</td>
<td>28±2^*</td>
<td>25±1^*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spontaneous VT (%)</td>
<td>60</td>
<td>15*</td>
<td>15*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VT duration (s)</td>
<td>12±3</td>
<td>3±1*</td>
<td>4±1*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Area at risk (%)</td>
<td>42±5</td>
<td>47±7</td>
<td>41±5</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Subacute myocardial infarction (dog)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>QRS (ms)</td>
<td>56±1</td>
<td>46±1*</td>
<td>51±2*</td>
<td>46±2*</td>
<td>56±1</td>
<td>47±2*</td>
<td>44±1*</td>
</tr>
<tr>
<td>EG duration, EBZ (ms)</td>
<td>30±1</td>
<td>23±3*</td>
<td>24±1*</td>
<td>21±1*</td>
<td>32±2</td>
<td>26±1*</td>
<td>21±2*#</td>
</tr>
<tr>
<td>QRS during PES from EBZ (ms) †</td>
<td>96±3</td>
<td>77±2*</td>
<td>80±4*</td>
<td>67±3*~</td>
<td>81±2</td>
<td>78±2</td>
<td>71±2*#</td>
</tr>
<tr>
<td>PES-induced VT/VF (%)</td>
<td>68</td>
<td>17*</td>
<td>43</td>
<td>80</td>
<td>70</td>
<td>38</td>
<td>70</td>
</tr>
<tr>
<td>Predominant arrhythmia morphology</td>
<td>polymorphic</td>
<td>polymorphic</td>
<td>monomorphic</td>
<td>polymorphic</td>
<td>polymorphic</td>
<td>polymorphic</td>
<td>polymorphic</td>
</tr>
<tr>
<td>Infarct size (%)</td>
<td>29±2</td>
<td>29±3</td>
<td>37±2*</td>
<td>33±2</td>
<td>29±2</td>
<td>28±2</td>
<td>28±3</td>
</tr>
<tr>
<td><strong>Subcellular location of the applied therapy</strong></td>
<td>Concentrated at intercalated disk</td>
<td>Concentrated at intercalated disk</td>
<td>Concentrated at intercalated disk</td>
<td>Random and not at intercalated disk</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Control mice were injected with Ad-empty, control dogs were injected with Ad-GFP in comparisons with the viral approaches or remained uninjected (sham) or received unmodified stem cells (cMSC) in comparison to stem cell delivery of SkM1. †: QRS duration averaged during baseline, S1, and S2 programmed electrical stimulation (PES) from the epicardial borderzone (EBZ). *: P<0.05 vs respective control. ^: P<0.05 vs baseline. #: P<0.05 vs cMSC. ~: P<0.05 vs Ad-SkM1 and Ad-Cx32.
Ischemia-reperfusion injury is quantified by the area that is at risk for infarction if there were complete coronary occlusion. Infarct models are typically quantified for their size, expressed as percentage of the total heart. Assessing the outcomes in Table 1, Cx32 overexpression increases infarct size in mice and dogs.\textsuperscript{22, 28} A likely explanation for this finding is that improved gap junction function may facilitate the spread of mediators of injury, thereby increasing the area of damage. Combined overexpression of SkM1/ Cx32, on the other hand, did not significantly increase infarct size. The latter may relate to less efficient Cx32 production, while another protein targeted to membrane compartment is also being overexpressed (SkM1 in this case).\textsuperscript{22} Also, the stem cell interventions tested did not significantly alter infarct size.\textsuperscript{26} In the ischemia-reperfusion model, there was no difference among the various strategies tested. The fact that Cx32 does not increase the area at risk in this setting presumably relates to the relatively short period of ischemia (5 min).\textsuperscript{18}

Final factors to consider are the application site, distribution and subcellular location of the various therapies. In the murine studies, single injections were made into the anterior left ventricular wall. This procedure resulted in transgene expression throughout both ventricles with higher protein levels in left vs right ventricle.\textsuperscript{18} In the canine studies, constructs (viral or stem cells) were injected into selected wide electrogram sites of the EBZ. Immunohistochemistry and Western blotting detected protein expression at the injection site, but not in the uninjected EBZ or elsewhere in the heart. With the viral approaches, we detected clear membrane staining of SkM1 and Cx32 and confocal images suggested a potential concentration of this expression at the intercalated disk.\textsuperscript{22} In contrast, SkM1-expressing cMSCs were randomly distributed through the EBZ and did not form intercalated disks.\textsuperscript{26}

I will now consider why some strategies did not generate an antiarrhythmic effect. In infarcted mouse and dog, Cx32 did improve gap junction communication but was not antiarrhythmic. A confounding factor in this outcome appears to be the larger infarcts in Cx32-treated animals.\textsuperscript{22, 28} In ischemia-reperfusion mice, Cx32 treatment was not associated with a larger area of risk and in this setting protection against arrhythmias was comparable to SkM1.\textsuperscript{18} Another interesting finding was the phenotype of the arrhythmia in canines treated with Cx32. Here, improved conduction in combination with larger (possibly more homogeneous) infarcts resulted in the occurrence of monomorphic rather than polymorphic arrhythmias (Figure 1). In SkM1/ Cx32, infarct size remained comparable to control and the arrhythmia converted back into a polymorphic phenotype. However, the finding that SkM1/Cx32 in this setting was not antiarrhythmic was surprising. In evaluating this outcome, it is important to realize that SkM1/Cx32 provided efficient means for restoring impulse propagation in the EBZ. Yet, the shorter QRS duration during premature stimulation in SkM1/ Cx32 vs SkM1 and Cx32 suggests the possibility of novel pathway formation. If these pathways support reentry and otherwise would have remained silent, they could now oppose the antiarrhythmic effect of speeding conduction (Figure 1).\textsuperscript{22} A comparable mechanism may have prevented cMSC/SkM1 from being antiarrhythmic (Figure 1).
Another problem with the cMSC/SkM1 approach is that a subcellular location of SkM1 at the intercalated disk was not demonstrable. Suboptimal distribution of the channel within the cell may have contributed to the failure to prevent arrhythmias.

In sum, the above-discussed combination of studies indicates that, although speeding conduction in suppressed areas may be antiarrhythmic, the following aspects need to be evaluated before therapies are applied: 1) the risk that increased gap junction function increases myocardial damage, and 2) the risk of improving conduction in reentrant pathways that otherwise would remain silent. Finally, as compared to stem cells, viral delivery appears at this stage superior for the delivery of antiarrhythmic genes aimed at improving conduction.

OTHER GENE-BASED THERAPIES TARGETING MYOCARDIAL INFARCTION RELATED ARRHYTHMIAS

In addition to attempts that speed conduction and prevent reentry, other gene and cell therapy strategies have been investigated to prevent myocardial infarction-related arrhythmias. These can be divided into strategies that prolong refractoriness, restore Ca²⁺-homeostasis, reduce myocardial damage or promote recovery from infarction.

Prolonging refractoriness

The use of class III antiarrhythmics (drugs like dofetilide that prolong AP duration, i.e., blockers of the delayed rectifier outward K-current) is largely hampered by their global effects on repolarization. The latter results in a lengthening of the QT interval which is associated with increased occurrence of *torsade de pointes* VT. To overcome the limitations of interfering with global repolarization, Donahue and co-workers developed a targeted approach for K-channel blockade.³³ They selected the dominant negative HERG mutant G628S, known for its association with the long QT syndrome, and introduced it into 4-week old porcine infarcts which had reproducible monomorphic VT. Transgene delivery was localized to the infarcted area via endovascular adenovirus infusion at the site of the initial balloon occlusion that had been used to create the infarct. One week after vector infusion, all HERG-G628S-treated animals showed prolonged ERP and absence of inducible arrhythmias. In contrast, VT inducibility remained unchanged in all control animals. Of note, the K-channel blocker dofetilide that was also tested in this model generated a prolongation of ERP and QTc, increased dispersion of the QT interval, and failed to prevent VT induction. A concern with the HERG-G628S strategy is that an inappropriately targeted prolongation of AP duration may be associated with triggered activity and *torsade de pointes* VT. At present, this remains a theoretical concern only, as proarrhythmia was not noted in this study.

As an alternative strategy to prolong AP duration, refractoriness may also be enhanced in the early post AP-repolarization phase. In this respect cellular delivery of Kv1.3 and Kir2.1 have both been demonstrated to prolong ERP while shortening AP duration.³⁴, ³⁵ Advantages of such an approach could be the positive impact on
conduction velocity in depolarized tissue and the suppressive effect on potentially proarrhythmic automaticity. However, a significant downside appears to be the very high cell-to-myocte ratio needed to obtain the desired effect. Because the present studies were conducted in healthy tissue, it remains to be seen to what extent sufficient numbers of cells survive within or around the myocardial infarcts. Furthermore, potential protection against inducible arrhythmias has not yet been investigated.

Restoring Ca$^{2+}$-homeostasis
Both clinical and experimental studies indicate that contractile dysfunction in heart failure is associated with reductions in expression or function of the sarcoplasmic reticulum (SR) Ca$^{2+}$ ATPase (SERCA). During membrane depolarization of myocardium, Ca$^{2+}$ entry into the cell triggers the SR to release Ca$^{2+}$ (Ca$^{2+}$-induced Ca$^{2+}$ release), which in turn initiates myofilament contraction. Relaxation is subsequently facilitated by removal of cytosolic Ca$^{2+}$ in which SERCA plays a major role. These observations led to the concept that SERCA2a gene transfer might improve contractile dysfunction in heart failure. Moreover, it has been shown that adeno-associated virus (AAV) delivery of SERCA2a in end-stage human heart failure is safe. Furthermore, a phase II trial also suggests important benefit with regard to the 6-minute walk test, left ventricular end-systolic volume, and time to clinical deterioration, meriting evaluation in larger trials. These improvements appear to provide strong support for the notion that restoration of suppressed SERCA levels improves both myocardial contraction and relaxation. In addition, SERCA overexpression appears to restore endothelial nitric oxide synthase expression, thereby improving myocardial perfusion and metabolism.

The antiarrhythmic potential of SERCA2a overexpression has been investigated in various settings. Initial studies of ischemia/reperfusion indicated reductions in infarct size and ventricular arrhythmias in SERCA2a-treated rats. Similar results were obtained in a porcine model of ischemia/reperfusion. In pigs with permanent coronary artery occlusion, infarct size remained unchanged and arrhythmias were not significantly altered. Lyon and coworkers noted reduced spontaneous non-sustained ventricular arrhythmias and fewer isoproterenol-induced episodes of VT/VF in rats in which SERCA2a was overexpressed in healed myocardial infarcts. Cellular studies indicated that SERCA2a reduced spontaneous SR Ca$^{2+}$ release, SR Ca$^{2+}$ leak and catecholamine-induced triggered arrhythmias. Reduced triggered arrhythmias may also result from restoration of Na$^+$/Ca$^{2+}$ exchanger function through normalization of miR-1 levels. Finally, in normal and failing guinea pig hearts, SERCA2a not only protects against triggered arrhythmias, but also reduces arrhythmogenic alterations in AP duration (i.e., AP alternans).

Reducing myocardial damage
Protection against myocardial infarction has been an initial focus of cardiovascular gene therapy. Growth factors and related molecules that stimulate angiogenesis have been proposed to increase collateral formation and decrease the susceptibility to major coronary artery occlusion. Here, vascular endothelial growth factor (VEGF)
gene therapy has been studied extensively in experimental and clinical settings. Clinical benefit from VEGF-based strategies has not convincingly been demonstrated, and prevention of arrhythmias has not been a primary goal in these cardioprotective gene therapies. However, some encouraging results have been obtained.

One approach includes myocardial overexpression of kallikrein which significantly protected against ischemia/reperfusion-induced cell death and ventricular arrhythmias in rats. Kallikrein is an upstream component of the kallikrein/kinin system which then activates the bradykinin B2 receptor resulting in vasodilation, increased vascular permeability, cell proliferation, and modulation of inflammation. The cardioprotective effects of angiotensin converting enzyme (ACE)-inhibitors also derive from inhibition of kinin degradation as ACE (kinase II) in itself is a kinin-degrading enzyme. Kallikrein gene therapy was originally developed as an alternative hypertensive treatment and has indeed been shown to reduce blood pressure and protect against end organ failure. In any case, the antiarrhythmic effect of kallikrein gene transfer appears to stem from a general protection against ischemia reperfusion-induced damage.

Myocardial protection and prevention of ventricular arrhythmias may also be obtained via gene transfer of hepatocyte growth factor (HGF). HGF is a multifunctional cytokine involved in migration, proliferation and tissue invasion of vascular endothelial and smooth muscle cells. In animal models of myocardial infarction, HGF gene transfer has enhanced angiogenesis and reduced apoptosis and fibrosis. The combination of these effects likely contributes to the increased VF induction threshold and reduced VF duration described in infarcted hearts in which HGF was overexpressed. Recently, HGF gene therapy has been evaluated in an open-label phase I clinical trial in which safety was demonstrated for naked DNA delivery of a combination of two HGF isoforms. Plasmid DNA was injected in 4-8 sites surrounding the posterior descending artery after off-pump coronary artery bypass grafting of the left coronary system. Significant improvements in global myocardial function (wall motion score and stress perfusion) were seen, which together with the demonstrated safety support the initiation of next-stage clinical trials for HGF gene therapy.

**MYOCARDIAL REGENERATION AND ARRHYTHMIAS**

Strategies that generate or direct myocardial repair may impact on post-infarction arrhythmias. Outcomes here are diverse, varying from significant pro-arrhythmia to moderate protection against non-sustained VT. It is generally accepted that efficient and uniform myocardial regeneration may reduce susceptibility to arrhythmias. Yet, depending on the cell type used, specific inhomogeneities may be introduced that may be proarrhythmic. Skeletal myoblast implantation exemplifies the latter situation: these cells generate skeletal myocytes which do not express cardiac connexins. Such cells remain uncoupled from adjacent host cardiac myocytes and are associated with significant arrhythmias. Although overexpression of Cx43 in skeletal myoblasts results in occurrence of coupling and reduces the proarrhythmic side effects, their inefficiency in restoring muscle contraction has largely disqualified these cells from further use.
Other cell types initially explored include those obtained from bone marrow (including mononuclear cells, MSCs, and others). Although these cells were originally considered a source for in vivo transdifferentiation toward cardiac myocytes, the emerging consensus is that they exert their regenerative effect via paracrine pathways, thus enhancing endogenous repair. The low numbers of cardiomyocytes formed in this process have not been associated with clinical arrhythmogenesis. However, there are potentially deleterious effects of the cells used to obtain this type of repair. In this respect, MSCs have been most extensively characterized. These cells express cardiac connexins but are unexcitable. Their membrane potentials are in the -30 to -40 mV range, which in vitro has been demonstrated to act as a current sink. Therefore, there is the potential for slow conduction and spiral wave arrhythmias. Yet, the poor-to-moderate engraftment rates of bone marrow derived cells possibly prevented such events from occurring in vivo. In fact, the incidence of ventricular arrhythmias may be slightly reduced by the application of bone marrow derived cells.

More recently, various groups have started to explore the use of cardiac progenitor cells. Different populations have been isolated including but not limited to cells that express c-Kit and Sca-1. Based on the experiences with non-cardiac progenitors, the rationale now was that these cells may generate direct myocardial regeneration, stimulate endogenous repair, or a combination of both. Initial studies indicate that both mechanisms are at play although the issue remains controversial. Phase I/II studies employing c-Kit positive cells or cardiosphere-derived cells (a mixture of c-Kit and Sca-1 positive cells) confirm safety in human subjects. However, due to the absence of arrhythmias at baseline, it remains uncertain to what extent this regeneration may be meaningful in terms of arrhythmia protection. Furthermore, the beneficial outcomes in terms of reduced infarct size, increased viable mass and improved ejection fraction should be interpreted with caution as no placebo groups were included (due to the invasive nature of these studies) and sample sizes were small.

Finally, stem cells may also be coaxed into a lineage of cardiac myocytes ex vivo and transplanted as such in an effort to improve myocardial function and reduce arrhythmias. Proof-of-concept for this type of an approach has been provided with embryonic cardiomyocytes and human embryonic stem cell-derived cardiomyocytes (hESC-CMs) transplanted in cryolesioned mice and guinea pigs, respectively. Both studies employed immunosuppression protocols to prevent rejection and used genetically encoded fluorescent calcium sensors to follow electrophysiological behavior of the transplanted cells. Another similarity was the finding that the transplanted cardiomyocytes improved ejection fraction and reduced PES-induced VT. The guinea pig study was of particular interest as it indicated that approximately 60% of the treated animals had electrically integrated grafts. Within these animals, 65-90% of the grafts were coupled to their host myocardium while the remaining grafts remained uncoupled and harbored spontaneous activity, thereby revealing the immature nature of the cells used. In sum, this second study showed that, despite incomplete electrical integration and apparent cellular immaturity, post myocardial
Infarction contractile dysfunction may still safely be improved. It remains however to be seen how these effects persist over time, and how the approach translates to larger animals with more realistic models of myocardial ischemia and infarction.

**ISSUES AND OPPORTUNITIES ON THE ROAD AHEAD**

As the field of antiarrhythmic gene and cell therapy advances, some selected approaches are emerging as promising pre-clinical candidates, while others, not primarily designed as antiarrhythmics, have reached to the level of early stage clinical testing (Table 2). Much is still to be learned about the underlying mechanisms of action and about means to identify patients who are most likely to benefit from selected therapies. More comprehensive testing in various cardiac pathologies will be important to evaluate the risk-benefit ratio and its contextual dependence. In the meantime, valuable information will be generated by application of selected therapies in ongoing and future clinical investigations.

Individual strategies will have their own hurdles that need to be dealt with. For example, approaches that employ transplantation of stem cell-derived cardiac myocytes need to be evaluated in light of cell sources that have more realistic clinical

### Table 2. Major strategies to prevent myocardial infarction related arrhythmias and most promising examples.

<table>
<thead>
<tr>
<th>Strategies &amp; examples</th>
<th>Potency to protect against arrhythmias</th>
<th>Phase of development</th>
<th>Refs.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Speeding conduction</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SkM1 GT</td>
<td>High</td>
<td>Pre-clinical, large animal</td>
<td>18, 20, 22, 25, 27</td>
</tr>
<tr>
<td>Connexin GT (Cx32 or Cx43)</td>
<td>Moderate</td>
<td>Pre-clinical, large animal</td>
<td>18, 19, 22, 28</td>
</tr>
<tr>
<td>miR-1 GT</td>
<td>Moderate</td>
<td>Pre-clinical, small animal</td>
<td>21</td>
</tr>
<tr>
<td><strong>Prolonging refractoriness</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HERG-G628S GT</td>
<td>High</td>
<td>Pre-clinical, large animal</td>
<td>33</td>
</tr>
<tr>
<td>Kv1.3 or Kir2.1 CT</td>
<td>Low-moderate</td>
<td>Pre-clinical, large animal</td>
<td>34-36</td>
</tr>
<tr>
<td><strong>Restoring Ca(^{2+}) homeostasis</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SERCA2a GT</td>
<td>Moderate</td>
<td>Clinical, Phase III</td>
<td>38-46</td>
</tr>
<tr>
<td><strong>Reducing myocardial damage</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kallikrein GT</td>
<td>Moderate</td>
<td>Pre-clinical, small animal</td>
<td>49</td>
</tr>
<tr>
<td>HGF GT</td>
<td>Moderate</td>
<td>Clinical, Phase II</td>
<td>53-56</td>
</tr>
<tr>
<td><strong>Myocardial regeneration</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bone marrow derived cells</td>
<td>Low</td>
<td>Clinical, Phase III</td>
<td>23, 61, 62, 66</td>
</tr>
<tr>
<td>Cardiac progenitor cells</td>
<td>Moderate</td>
<td>Clinical, Phase II</td>
<td>67, 68</td>
</tr>
<tr>
<td>iPSc-derived cardiac myocytes</td>
<td>Moderate</td>
<td>Pre-clinical, small animal</td>
<td>77</td>
</tr>
</tbody>
</table>

GT: Gene therapy. CT Cell therapy. See text for details.
potential. Autologous induced pluripotent stem cells (iPS) may be considered, these cells still have many issues of their own (including the oncogenic risks associated with inducing a pluripotent state and the heterogeneity in the cellular phenotypes that are subsequently generated). In addition, the HERG-G628S and SkM1 strategies would likely benefit from the incorporation into long-term expression vectors such as AAV and lentiviral vectors, respectively (SkM1 is too large for incorporation into AAV). Development of these types of vectors will also facilitate investigation of potentially beneficial or deleterious effects of long-term transgene overexpression. Another issue for the HERG-G628S and SkM1 approaches is delivery to opportune sites. Here, clinically available endocardial and epicardial mapping techniques may need to be optimized for substrate detection and catheter or open-chest delivery of the constructs involved. It is expected that over time goals like long-term stem cell maintenance and stable myocardial transgene expression will be achieved, while generating meaningful therapeutic benefit. This will also create significant opportunities for protection against non-ischemic cardiac arrhythmias. The development of biological pacemakers is one such an example, but also protection against atrial fibrillation may be achieved. Furthermore, genetic correction of inherited arrhythmia syndromes will be another area of interest. Early signs of success have been noted, and with the arrival of techniques that facilitate the development of large animal models of these lethal syndromes, we are set to explore the full potential of molecular-defined antiarrhythmics.

CONCLUSIONS

The development of gene and cell therapy is a work in progress. Many of the conceptualized strategies are still in pre-clinical testing and have only been validated in small-sample studies. Yet, these novel molecular interventions are providing opportunities to locally modify the cardiac substrate, modify specific genes or pathways, and regenerate the cardiac muscle, all to a degree that has been impossible with classical pharmacology. These novel therapies therefore have significant potential to reduce the disease burden of life-threatening cardiac arrhythmias.

REFERENCES

7. Durrer D, Roos JP. Epicardial excitation of the ventricles in a patient with Wolff-


51. Martorana PA, Kettenbach B, Breipohl G, Linz W, Scholkens BA. Reduction of infarct size by local angiotensin-converting enzyme inhibition is abolished by a


57. Reinecke H, Poppa V, Murry CE. Skeletal muscle stem cells do not transdifferentiate into cardiomyocytes after cardiac grafting. *J Mol Cell Cardiol* 2002; 34:241-249.


