Cardiac arrhythmias are a major healthcare problem in the developed world. The American Heart Association 2017 statistical report indicates that atrial fibrillation, the most common cardiac rhythm disorder, has an ≈25% lifetime incidence and annual costs of ≈26 billion dollars, whereas sudden cardiac death (usually caused by malignant arrhythmias) affects fewer individuals (just under 200,000 Americans/year) but has more disastrous consequences.1 There have been a variety of improvements in arrhythmia therapy over the past decades, particularly in the realm of nonpharmacological approaches, but many challenges remain.2

Allele-Specific Gene Silencing to Treat CPVT

In the present issue of Circulation Research, Bongianino et al6 present the results of an elegant and highly novel approach to treating autosomal-dominant forms of CPVT with the use of targeted allele-specific gene silencing. The authors aimed to use short-interfering ribonucleic-acid (siRNA) to specifically suppress the expression of a mutant ryanodine-receptor type-2 allele bearing a dominant negative (R4496C) mutation to treat CPVT in a well-established mouse model. siRNAs are short (≈20–24) nucleotide strands complementary to specific sequences in the target mRNA: incorporated in the endogenous RNA-silencing complex, they prevent gene expression by blocking translation, promoting mRNA-breakdown, and causing mRNA cleavage.

The authors first developed an efficient in vitro screening assay to identify candidate siRNAs and compare their ability to target the mutant allele specifically, with minimal affinity for the wild-type gene. They then inserted the optimally selective siRNA sequence into an RNA interference vector, including the cytomegalovirus promoter, to drive efficient and long-term expression of the siRNA and a GFP (green fluorescence protein) reporter. The vector was then packaged into an AAV9, with a similarly prepared AAV containing a scrambled microRNA sequence in place of the siRNA acting as a control. Mice that were heterozygous for the dominant-negative R4496C mutation were injected intraperitoneally with siRNA or scrambled-sequence AAV9 at days 8 and 30 after birth and studied 8 weeks later.

AAV bearing siRNA (AAV/siRNA) strongly suppressed the induction of bidirectional ventricular tachycardia by intraperitoneal epinephrine (20 mg/kg) and caffeine (120 mg/kg). In parallel, while cardiomyocytes from mice injected with scrambled-sequence AAV9 or GFP-negative cardiomyocytes from AAV/siRNA-injected mice showed a high prevalence of delayed afterdepolarizations and triggered activity on challenge with 30 mM/L isoproterenol, these were virtually absent in GFP-positive cardiomyocytes from AAV/siRNA-injected mice. Mutant mice showed structural remodeling of...
mitochondria, which was attenuated in AAV/siRNA-injected mice. Interestingly, these dramatic improvements were noted with relatively modest target-allele knockdown: mRNA expression of the mutant allele was reduced by only 38% in AAV/siRNA-injected mice. The authors estimated that AAV/siRNA treatment increased the proportion of completely wild-type ryanodine-receptor type-2 tetramers from 6.25% in heterozygous mice to 19.75%, while reducing completely mutant tetramers from 6.25% to 1.23% and 3-mutant/1-wild-type tetramers from 25% to 9.87%.

**Significance and Implications of the Findings**

The Bongianino study presents several novel and important findings. The authors demonstrate the feasibility of allele-specific silencing as a treatment for autosomal-dominant inherited cardiac diseases. The possibility of knocking down a pathogenic allele without affecting the fully functional wild-type allele is an exciting prospect. The use of siRNA allows for the delivery of relatively small therapeutically active nucleotide sequences, well within the capacity of most viral vectors. The combination of the cardiotropic AAV vector and the efficient cytomegalovirus promoter permitted long-lasting, cardiomyocyte-selective expression in mice (whether the same can be achieved in man remains to be seen). Furthermore, the study showed that even moderate target-allele knockdown can provide substantial antiarrhythmic efficacy.

This work is an exciting development because it provides a proof of the principle that dominant-negative inherited arrhythmia syndromes may be susceptible to specific and highly effective therapy via a single parenteral injection of the appropriate therapeutic construct. There are many potentially life-threatening forms of congenital arrhythmia that show autosomal-dominant inheritance, including most cases of Long-QT Syndrome, CPVT, Brugada Syndrome, Short-QT Syndrome, and arrhythmogenic cardiomyopathy. Drug therapy is available for only some of these conditions (eg, β-blockers for Long-QT Syndrome and CPVT, flecainide for CPVT, quinidine for Brugada and Short-QT Syndrome) and is often only partially effective. While implantable devices are lifesaving and widely used in patients with inherited arrhythmias, they are fraught with potential complications, have a limited life span, sometimes require the use of adjuvant drug therapy because of frequent discharges, and can be complicated by electrical storm. The feasibility of providing effective and persistent AAV-delivered cardiac gene therapy in mice has been demonstrated. Although a large clinical trial in severe heart failure was negative, this was likely because of technical problems, some of which were related to the challenges of delivering a large gene (encoding the sarcoplasmic-reticulum Ca²⁺-ATPase). Single subcutaneous doses of an siRNA targeting PCSK9 (proporionate convertase subtilin-kenix type-9) dramatically reduce low-density lipoprotein cholesterol for >3 months, indicating the long-lasting effectiveness of siRNA therapy in man. Thus, it is conceivable that appropriately designed allele-silencing siRNA-based therapeutics could provide reliable, stable, and safe arrhythmia protection to many individuals with potentially lethal inherited arrhythmia syndromes, replacing ineffective antiarrhythmic drugs and expensive, potentially complicated implanted devices.

**Limitations and Possibilities**

While it is important to dream, it is also important to recognize the practical limitations to the clinical application of allele-silencing therapy. A major concern is the need to carefully design sequence-specific siRNAs for each dominant-negative mutation of each congenital arrhythmia syndrome. There are potentially thousands of such distinct mutations, each affecting a relatively small number of patients. The need to produce and properly test specific therapeutic constructs for each of the mutations, with a potential market of only a few individuals, is economically daunting. Perhaps, at some point in the future it will be possible to create and thoroughly test a template delivery system and prototype siRNA (so that new siRNAs could be efficiently tested in vitro for efficacy and then simply inserted into a standardized optimized template) to streamline the development and application process.

A second concern is the need to ensure selective and effective cardiac delivery. In the Bongianino study, this goal was achieved by intraperitoneal delivery of cardiotropic AAV to mice. However, cell-selectivity of AAVs varies among species, and it may not be possible to use the same strategy in man. In the CUPID-2 trial (Calcium Uregulation by Percutaneous Administration of Gene Therapy in Patients With Cardiac Disease), an AAV carrying the therapeutic construct was injected directly via a coronary artery. This technique provides direct cardiac exposure, but the rapid transit time through the heart limits the selectivity attainable by coronary injection. The common presence of neutralizing antibodies to AAVs is another reality that may limit the usefulness of AAV delivery in many individuals. Inclisiran, the synthetic PCSK9 siRNA used for cholesterol lowering, is conjugated to carbohydrate groups that bind to abundant liver-expressed asialoglycoprotein receptors, allowing for strong hepatocyte uptake without the need for a viral delivery vector. Perhaps, a modified version of this method could be used to provide cardiomyocyte-specific molecular targeting.

A final issue is the limited applicability of allele-silencing therapy. The size of the congenital arrhythmia population is small, not all cases involve autosomal dominant inheritance, and not all of the dominantly inherited mutations will be amenable to siRNA-mediated allele silencing. Ultimately, it would be interesting to apply gene silencing to treat the much more common forms of cardiac arrhythmia that are not caused by monogenic inherited syndromes. Highly selective siRNA-mediated silencing of genes encoding ion-channel subunits or other proteins critical to arrhythmia pathogenesis might eventually offer safe and effective therapeutic approaches to widespread and problematic arrhythmias like atrial fibrillation or the malignant ventricular tachyarrhythmias that cause sudden cardiac death. The major challenge in this area is the identification of appropriate therapeutic targets. The definition of novel molecular targets suitable for arrhythmia treatment is a subject of intensive ongoing investigation but remains a challenge.

Despite all of the reservations noted earlier, the successful development by Bongianino et al of an allele-silencing approach that virtually eliminates CPVT in a clinically relevant
animal model is an exciting and significant advance. When one considers the progress that has been achieved in cardiac gene-therapy approaches over the 17 years since Donahue et al first reported the ability of viral gene transfer to slow the ventricular heart rate in atrial fibrillation, it seems inevitable that in the foreseeable future, we will see the successful clinical application of gene therapy to treat cardiac arrhythmias and that this will eventually revolutionize arrhythmia management.

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References

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Stanley Nattel

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