Is stem cell therapy proarrhythmic?

**Stem Cells Are Not Proarrhythmic**

Letting the Genie out of the Bottle

Hung Q. Ly, MD, MSc; Stanley Nattel, MD

Ischemic heart disease remains a leading cause of mortality worldwide. Myocardial infarction (MI) accounts for more than half of cardiovascular-related deaths.1 With rising numbers of surviving MI patients and an aging population, congestive heart failure has become a major health concern, with symptomatic patients having a poorer prognosis.2 Despite substantial advances in medical and device strategies for congestive heart failure, there remains a growing unmet clinical need. Accordingly, various novel therapies have been explored in recent years, with the potential offered by stem cells (SCs) arguably garnering the most attention.3,4 Cardiac SC therapy has emerged as a promising option to repair ischemic/dysfunctional myocardium through a variety of potential mechanisms (Figure 1).5–7 Continuing debates over unresolved mechanistic issues and the rapid transition toward clinical application, with already-demonstrated modest but tangible benefits for cardiac structure and function, underscore the fact that the field is still in its infancy.8–10 As with other emerging therapies, safety concerns have arisen in relation to potential hazards of SC therapy.11

Among the most contentious issues, the risk of proarrhythmia has sparked debate and generated lingering doubts, which run the risk of paralyzing a potentially revolutionary opportunity.12–15 Although findings that have raised concerns about proarrhythmic potential of SC transplants cannot be discounted, we contend that the available data are encouraging rather than damning. Here, we highlight experimental and clinical data on SC-related arrhythmogenesis, discuss possible confounding issues, and argue that SC therapy, rather than being considered intrinsically proarrhythmic, must be understood to have controllable proarrhythmic risks along with unique antiarrhythmic potential.

Are SCs Intrinsically Proarrhythmic?

Initial clinical trials involved satellite cells or skeletal myoblasts (SkMs)16,17 and suggested proarrhythmic potential,17 despite a lack of proarrhythmia in early preclinical studies.18,19 In the first phase I clinical trial using autologous SkMs, Menasché et al selected patients eligible for coronary artery bypass grafting (CABG), with severe left ventricular dysfunction (left ventricular ejection fraction [LVEF] ≥35%) and akinetic scars, to undergo multiple transepicardial cell injections to deliver an average of 87×10⁶ cells.17 Clinically significant ventricular arrhythmias were later documented in 4 of the 9 patients included in this study (3 of whom had a history of significant prior ventricular arrhythmia), which led to the mandatory use of automatic implanted cardioverter-defibrillators. Subsequently, other trials investigating the adjunctive use of autologous SkMs in patients with chronic ischemic cardiomyopathy undergoing surgical revascularization also reported postoperative ventricular tachyarrhythmias (VTs), despite notable differences in the number of cells delivered at the time of surgery (from 0.2×10⁷ to 30×10⁷).20–22 With a percutaneous intramyocardial route, Smits et al reported proarrhythmic effects of autologous SkMs in postinfarction patients with depressed LVEF.23 Similarly, the use of bone marrow–derived cells (BMCs) has been linked, albeit to a lesser degree, with increased occurrence of VT. Hendrix et al...
randomized 20 elective CABG patients to receive either heparinized saline or multiple intramyocardial injections of CD34+ BMCs in the border zone of a nonviable scar (as assessed by magnetic resonance imaging and thallium scintigraphy). Follow-up programmed ventricular stimulation studies were performed 6 to 8 weeks postinjection. Six of 9 BMC patients were found to have inducible VT (5 monomorphic, 1 polymorphic) compared with none of 5 controls. These preliminary reports pointed to an alarmingly higher-than-expected incidence of VTs associated with SC therapy versus contemporary post-MI subjects.

The first wave of clinical studies primarily focused on contractile benefits of adult SCs and their potential for cardiac regeneration. Parallel experimental data began to shed light on the electrophysiological impact of SC cardiomyocyte interactions.

Although cell engraftment occurs, nested SkMs remain electrically isolated from surrounding myocardium. The failure of electromechanical coupling is attributable to insufficient gap junction formation with host tissue. The absence of expression by SkMs of connexin-43, the predominant gap-junction channel–forming protein in mammalian ventricular tissue, has been identified as the cause for poor functional integration. Undifferentiated SkMs express large amounts of this protein, as well as N-cadherin, but as SkMs differentiate into myotubes, these key gap junction proteins become markedly downregulated. Cocultured rat cardiomyocytes and human SkMs demonstrate arrhythmogenic spiral waves that are suppressed by engineering connexin-43–expressing SkMs for coculture.

Human mesenchymal stem cells (MSCs) elicit reentrant arrhythmias on coculture with neonatal rat ventricular cardiomyocytes despite good coupling, presumably because of increased tissue heterogeneity induced by inexcitable human MSCs coupled to neonatal rat ventricular cardiomyocytes. Pak et al investigated the effect of injecting human MSCs and BMCs on cardiac innervation in a swine infarction model. Relative to control groups, pigs injected with a combination of BMCs and MSCs had greater densities of developing nerves staining positive for tenascin, a family of extracellular matrix proteins linked to cardiovascular remodeling and nerve regeneration. The investigators speculated, on the basis of prior studies linking cardiac hyperinnervation with sudden cardiac death, that heterogeneous sympathetic nerve sprouting imparted arrhythmia susceptibility. Finally, Zhang et al uncovered the potential for all 3 classic proarrhythmia mechanisms (reentry, triggered activity, and automaticity) in whole-cell patch-clamp studies of ESC-derived cardiomyocytes.

### Shades of SC Proarrhythmia

The current perception of the proarrhythmic potential of SC may have been skewed by early disquieting reports from SkM studies in chronic ischemic cardiomyopathy. A summary of clinical trials data relating to the proarrhythmic potential of SC therapy is provided in Table 2. Without discounting these studies, it is crucial to consider whether the proarrhythmic risk is preventable or controllable (or both), whether the risk is transient, and, finally, whether the risk is shared by all candidate SC treatment approaches to cardiac repair.

It is very important to remember the inherent arrhythmic substrate of patients enrolled in the initial SkM trials. Diseased ischemic myocardium represents an ideal setting for ventricular arrhythmogenesis based on preexisting (anatomic and electrophysiological) heterogeneities, as well as dynamic instability manifested by electrical alternans, conspiring to...
amplify any proarrhythmic risk. Although VT is rather uncommon among CABG patients, specific subsets of patients are particularly at risk. The combination of prior MI, severe symptomatic heart failure, and low left ventricular ejection fraction (≤40%) identifies CABG patients with a 30% likelihood of developing VT, versus 1% when none are present. 

Although a firm causal relationship between SC therapy and VT is difficult to ascertain from the initial, nonrandomized, and noncontrolled studies, heightened awareness of proarrhythmic risk associated with SkMs led investigators to introduce prophylactic amiodarone therapy at the time of cell transplantation. Herreros et al administered oral amiodarone for 3 months to elective CABG patients (mean LVEF, 36%) injected subepicardially with SkMs into akinetic or dyskinetic tissue deemed nonviable by stress echocardiographic and positron emission tomography studies. At 1-year follow-up, beneficial effects on contractility and viability were documented without occurrence of VT. Similarly, Siminiak et al administered prophylactic amiodarone in 2 phase I (surgical and percutaneous) trials. After VT episodes were observed early after SC transplantation in the first 2 study patients, all remaining CABG patients undergoing SkM injections were given amiodarone up to 6 weeks postintervention. No subsequent arrhythmic events were documented. These investigators then undertook the POZNAN trial in chronic ischemic cardiomyopathy (LVEF 25% to 40%), with percutaneous intramyocardial SkM delivery to nonviable tissue, supplemented with 2 to 3 weeks of oral amiodarone. Throughout the 6-month follow-up period, patients receiving this relatively short course of amiodarone did not experience VT. These data suggest that there may be a transient early

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**Table 1. Electrophysiological Mechanisms and Effects of Stem Cells**

<table>
<thead>
<tr>
<th>Proarrhythmic Effect</th>
<th>Antiarrhythmic Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Intrinsic electrophysiological properties</strong></td>
<td>Generation of islands of spontaneous current (biopacemakers)</td>
</tr>
<tr>
<td>Induces automaticity</td>
<td>Reduces arrhythmia inducibility</td>
</tr>
<tr>
<td>Predisposes to early and delayed afterdepolarization (triggered activity)</td>
<td>Improves electrical viability</td>
</tr>
<tr>
<td>Reduces conduction velocity</td>
<td>Can improve LV synchrony</td>
</tr>
<tr>
<td><strong>Graft-host tissue coupling</strong></td>
<td>Improves functional engraftment by gap junction formation</td>
</tr>
<tr>
<td>Limited cell-to-cell coupling leads to isolated cell clusters</td>
<td>Reestablishes impulse propagation</td>
</tr>
<tr>
<td>Reentry from anatomic or functional conduction blocks</td>
<td>Antitachyarrhythmic effect of selective localized conduction blocks</td>
</tr>
<tr>
<td><strong>Ion channel function</strong></td>
<td>Ex vivo expansion of gene-engineered SCs with ideal action potential phenotypes</td>
</tr>
<tr>
<td>Maturation and differentiation levels affect ion channel expression</td>
<td>Ex vivo selection of SC-derived cardiomyocytes with ideal ion channel expression for either impulse generation (biopacemaker) or improved engraftment</td>
</tr>
<tr>
<td>Dissimilar Na⁺ and K⁺ channel expression leads to prolongation of action potentials and refractoriness</td>
<td>Precondition SC to display “cardiac-like” ion-channel isoform expression</td>
</tr>
<tr>
<td><strong>Cardiac tissue heterogeneity</strong></td>
<td>Reduces heterogeneity by reducing ischemia and limiting infarct size. SC-derived cardiomyocytes or recruitment of resident cardiac SCs improves tissue homogeneity</td>
</tr>
<tr>
<td>Inhomogenous islands of nonexcited cells creating reentry pathways, potential source of triggered activity, and foci for automaticity</td>
<td>Improves ventricular remodeling</td>
</tr>
<tr>
<td>Elicited inflammatory reaction from SC apoptosis or profibrosis cytokine release</td>
<td>Diminishes wall stress</td>
</tr>
<tr>
<td>Increased sympathetic nerve sprouting</td>
<td>Improves regional contractility</td>
</tr>
</tbody>
</table>

**Figure 2.** Issues to consider when assessing the potential proarrhythmic effect of stem cells.
arrhythmic risk, possibly due to inflammatory reactions or irritative effects (or both) of intramyocardial injection. An additional potential consideration is the use of autologous serum for cell expansion and delivery. In contrast to prior studies, Herreros et al avoided xenogenic serum interaction with cultured SKMs, with an apparently reduced proarrhythmic profile. Chachques et al subsequently noted no malignant VT in 20 patients after transplantation of SKMs when the latter were expanded in the patients’ own serum.44

Menasché et al then initiated the multicenter, randomized, placebo-controlled Myoblast Autologous Grafting in Ischemic Cardiomyopathy (MAGIC) trial, a dose escalation study targeted in left ventricular remodeling was observed among high cell transplant number versus placebo patients. On the basis of blinded automatic implanted cardioverter-defibrillator interrogation, no death was attributed to arrhythmic events, although a non–statistically significant greater number of VTs in SKM-treated patients (n = 2, placebo group; n = 4, low cell group; n = 5, high cell group) was observed. Of note, no dose-response risk for proarrhythmia or time to first arrhythmic event was discovered.

Clinical trials using BMC transplants in the setting of recent MI (<14 days) have suggested functional benefits (eg, improved LV remodeling, reduction in infarct size). Varying results have been explained by differences in timing of administration or quality of cell harvest (or both), although sustainability of benefits remains controversial. Most BMC studies have focused on patients with preserved LVEF, and only a handful have investigated end-stage congestive heart failure patients or those not amenable to standard revascularization strategies. Whether administered by surgical or percutaneous methods, via intracoronary or intramyocardial routes, early or remotely after acute MI, using selected or unselected BMCs, or as a combination of BMC subtypes, BMC therapy studies have generally shown a lack of VT events as evaluated by serial 24-hour Holter monitoring or programmed simulation studies (or both).

In parallel, insights gained from experimental data have helped to refine our understanding of the proarrhythmic risk. These studies have confirmed a greater propensity for SKMs (compared with BMCs) to generate ventricular tachyarrhythmias, using optical mapping studies, Mills et al found that, while SKMs invariably induced arrhythmias following injection, engrafted MSCs preserved electrical viability, maintained impulse propagation along the border zone and showed decreased VT inducibility. Injection may be a key consideration: Fouts et al compared the injection of SKMs in scar tissue compared with neighboring viable regions and found increased VT-propensity after border-zone compared with central-scar injections. The route of delivery is also important. Fukushima et al found that when compared with direct intramyocardial injection, myoblast delivery via retrograde intracoronary injection produced more

### Table 2. Skeletal Myoblast Clinical Trials and Arrhythmic Side Effects

<table>
<thead>
<tr>
<th>Study (Year)</th>
<th>Study Type</th>
<th>Patients, n (Active/Control)</th>
<th>LVEF, Mean or Entry Criteria, %</th>
<th>Concomitant Procedure</th>
<th>Intramyocardial Delivery Route</th>
<th>Autologous Serum</th>
<th>Amiodarone Prophylaxis</th>
<th>Serious VT, Yes or No (n or Active/Control)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Menasché (2003)†</td>
<td>Nonrandomized</td>
<td>10 (10/0)</td>
<td>15–35</td>
<td>CABG</td>
<td>Transepicaldrial</td>
<td>No</td>
<td>No</td>
<td>Yes (4)</td>
</tr>
<tr>
<td>Pagani (2003)23</td>
<td>Nonrandomized</td>
<td>5 (5/0)</td>
<td>5–25</td>
<td>LVAD</td>
<td>Transepicaldrial</td>
<td>No</td>
<td>No</td>
<td>Yes (3)</td>
</tr>
<tr>
<td>Smits (2003)23</td>
<td>Nonrandomized</td>
<td>13 (13/0)</td>
<td>20–40</td>
<td>Catheterization</td>
<td>Endomyocardial</td>
<td>No</td>
<td>Yes</td>
<td>Yes (4)</td>
</tr>
<tr>
<td>Herreros (2003)41</td>
<td>Nonrandomized</td>
<td>12 (12/0)</td>
<td>≥25</td>
<td>CABG</td>
<td>Transepicaldrial</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Chachques (2004)44</td>
<td>Nonrandomized</td>
<td>20 (20/0)</td>
<td>≥25</td>
<td>CABG</td>
<td>Transepicaldrial</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Ince (2004)37</td>
<td>Nonrandomized</td>
<td>12 (6/6)</td>
<td>20–40</td>
<td>Catheterization</td>
<td>Endomyocardial</td>
<td>No</td>
<td>Yes</td>
<td>Yes (2)</td>
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<tr>
<td>Siminiak (2004)43</td>
<td>Nonrandomized</td>
<td>10 (10/0)</td>
<td>25–40</td>
<td>CABG</td>
<td>Endomyocardial</td>
<td>No</td>
<td>Yes</td>
<td>Yes (2)</td>
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<tr>
<td>Siminiak (2005)43</td>
<td>Nonrandomized</td>
<td>10 (10/0)</td>
<td>25–40</td>
<td>Catheterization</td>
<td>Transvenous</td>
<td>No</td>
<td>Yes</td>
<td>Yes (1)</td>
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<tr>
<td>Dib (2005)22</td>
<td>Nonrandomized</td>
<td>24 (24/0)</td>
<td>28</td>
<td>CABG-LVAD</td>
<td>Endomyocardial</td>
<td>No</td>
<td>No</td>
<td>Yes (3)</td>
</tr>
<tr>
<td>Dib (2007)*</td>
<td>Randomized</td>
<td>23 (12/11)</td>
<td>≤40</td>
<td>Catheterization</td>
<td>Endomyocardial</td>
<td>No</td>
<td>N/A</td>
<td>Yes (1/1)</td>
</tr>
<tr>
<td>Sherman (2008)†</td>
<td>Nonrandomized</td>
<td>20 (20/0)</td>
<td>20–40</td>
<td>Catheterization</td>
<td>Endomyocardial</td>
<td>No</td>
<td>No</td>
<td>Yes (4)</td>
</tr>
<tr>
<td>Menasché (2008)45</td>
<td>Randomized</td>
<td>97 (67/30)</td>
<td>15–35</td>
<td>CABG</td>
<td>Transepicaldrial</td>
<td>No</td>
<td>Yes</td>
<td>Yes (9/2)</td>
</tr>
</tbody>
</table>

homogeneous cell dissemination, less inflammation and disruption of myocardial architecture, and a decline in arrhythmic events.67 SCs can modulate action-potential duration via paracrine factors,31 but cardiomyocytes can also modulate ion channels in surviving SCs through paracrine effects, rendering them less arrhythmogenic. Zebedin et al found that SkMs exposed to cardiomyocyte-conditioned media show altered channel-isoform expression with “cardiac-like” Na-channel properties, chiefly mediated by upregulation of the cardiac isoform Na, 1.5.68 Halbach et al found that fetal cardiomyocytes engrafted at the border zone display “electrophysiological plasticity” and maturation in action-potential properties.69 Identifying the soluble factor(s) that can regulate electrophysiological properties of exogenous SCs might provide a key to improving functional integration and warrants further study.

**Potential Antiarrhythmic Effects of SC Therapy**

Any discussion of the proarrhythmic properties of SC therapy must not ignore the fact that SCs also have the potential to be antiarrhythmic (Figure 3). The most obvious antiarrhythmic component of SC therapy stems from its potential ability to lessen ischemia, limit infarct size, and favorably affect LV remodeling, thereby controlling endogenous arrhythmic substrates. Electrical heterogeneity and conduction disturbances could be attenuated by effective cardiac regeneration strategies, such as increased recruitment of resident cardiac SCs or grafting of cardiac SCs with suitable electrophysiological properties, or from the exciting possibilities emerging from reprogramming adult fibroblasts to induce SC properties.70,74 Experimental data using gene transfer to engineer SC function have shown promise with respect to increased survival and homing.76 SkMs overexpressing connexin-43 show reduced VT inducibility both in cocultured systems and in murine infarction models.31,77

Electrical coupling can occur between cardiomyocytes and nonmyocyte cells such as cardiac fibroblasts.78 Exogenous SCs could conceivably couple with host cardiac tissue through a span of a few nonmyocyte cells or even act as conduction bridges, given that variable degrees of structural coupling have been reported for both SkMs and MSCs.79

Far from “proarrhythmic,” SCs may have frank “prorhythmic” (antiarrhythmic) properties against both brady- and tachyarrhythmias. Engineered human MSCs overexpressing the pacemaker channel gene mHCN2 form gap junctions with surrounding host tissue and generate spontaneous rhythms.80 Another innovative alternative to creating biological pacemakers is to drive human ESC differentiation toward early-stage cardiomyocytes with pacemaker properties.81,82 Space does not permit a detailed discussion of SC-approaches to abolishing tachyarrhythmias, but the interested reader is referred to recent relevant articles.83,84 The possibilities opened by specific targeting of engineered SC therapy to arrhythmic tissues offer unparalleled potential for innovative ventricular antiarrhythmic therapy.85 Finally, clinical trials have begun to incorporate electrophysiological considerations of SC therapy to address the close relationship between electrical activation and ventricular function. After intracoronary delivery of unfractionated BMCs, Chang et al documented an impact of SC therapy not only on exercise capacity and infarct volume but also on LV systolic synchrony, as defined by the time to peak positive systolic velocity standard deviation, a reliable parameter of LV synchronicity in ischemic cardiomyopathy.86,87 These intriguing findings may provide novel alternatives for cardiac resynchronization therapy and will certainly be the object of future investigation.

**Conclusions**

In recent years, SC biology has produced a paradigm shift in therapeutic options for a multitude of cardiovascular diseases. By highlighting the electrophysiological implications of SC therapy, an awareness of proarrhythmic risk has helped to move this emerging field forward. Future studies will consider the intrinsic electrophysiological properties of SCs, track and modulate both physical and electrophysiological integration with cardiac tissue, and address any proarrhythmic potential. Ultimately, these efforts will pave the way to harnessing both the healing and antiarrhythmic potential of SC. Thus, it has become clear that it is no longer a question of if but of how the genie will be let out of the bottle.

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Disclosures

None.

References


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Response to Ly and Nattel

Ester Macia, MD; Penelope Boyden, PhD

We appreciate the review and comments of Drs Ly and Nattel on this issue. They state that stem cell therapy “must be understood to have controllable proarrhythmic risk.” Malignant arrhythmias are a major concern in patients with advanced heart disease. Although implanted cardioverter-defibrillators may be regarded as life vests for patients at increased risk of malignant arrhythmias, they should not serve as shields to support potentially arrhythmic treatments. The effectiveness and antiarrhythmic mechanism of amiodarone in this setting have not been addressed. It seems necessary to test amiodarone and other drugs in experimental models to understand their electrophysiological effects on the different stem cells and host properties. Drs Ly and Nattel also state that “transient early arrhythmic risk [is] possibly due to inflammatory reactions or irritative effects (or both) of intramyocardial injections.” Veltman et al (our ref 41) performed transendocardial transplantation of autologous skeletal myoblasts in postinfarction patients with severe left ventricular dysfunction. J Endovasc Ther. 2004; 11:695–704.


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