

This article is in a thematic series on **Recent Advances in Induced Pluripotent Stem Cell Research**, which includes the following articles:

Steps Toward Safe Cell Therapy Using Induced Pluripotent Stem Cells [*Circ Res* 2013;112:523–533]

Induced Pluripotent Stem Cells in Cardiovascular Drug Discovery [*Circ Res* 2013;112:534–548]

Immunogenicity of Pluripotent Stem Cells and Their Derivatives [*Circ Res* 2013;112:549–561]

Progress in the Reprogramming of Somatic Cells [*Circ Res* 2013;112:562–574]

Direct Cardiac Reprogramming: From Developmental Biology to Cardiac Regeneration [*Circ Res* 2013;113:915–921]

Perspectives for Induced Pluripotent Stem Cell Technology: New Insights into Human Physiology Involved in Somatic Mosaicism [*Circ Res* 2014;114:505–510]

Engineering Adolescence: Maturation of Human Pluripotent Stem Cell-derived Cardiomyocytes [*Circ Res* 2014;114:511–523]

**Induced Pluripotent Stem Cells for Post-Myocardial Infarction Repair: Remarkable Opportunities and Challenges**

*Shinya Yamanaka, Guest Editor*

## Induced Pluripotent Stem Cells for Post-Myocardial Infarction Repair Remarkable Opportunities and Challenges

Pratik A. Lalit, Derek J. Hei, Amish N. Raval, Timothy J. Kamp

**Abstract:** Coronary artery disease with associated myocardial infarction continues to be a major cause of death and morbidity around the world, despite significant advances in therapy. Patients who have large myocardial infarctions are at highest risk for progressive heart failure and death, and cell-based therapies offer new hope for these patients. A recently discovered cell source for cardiac repair has emerged as a result of a breakthrough reprogramming somatic cells to induced pluripotent stem cells (iPSCs). The iPSCs can proliferate indefinitely in culture and can differentiate into cardiac lineages, including cardiomyocytes, smooth muscle cells, endothelial cells, and cardiac progenitors. Thus, large quantities of desired cell products can be generated without being limited by cellular senescence. The iPSCs can be obtained from patients to allow autologous therapy or, alternatively, banks of human leukocyte antigen diverse iPSCs are possible for allogeneic therapy. Preclinical animal studies using a variety of cell preparations generated from iPSCs have shown evidence of cardiac repair. Methodology for the production of clinical grade products from human iPSCs is in place. Ongoing studies for the safety of various iPSC preparations with regard to the risk of tumor formation, immune rejection, induction of arrhythmias, and formation of stable cardiac grafts are needed as the field advances toward the first-in-man trials of iPSCs after myocardial infarction. (*Circ Res*. 2014;114:1328-1345.)

**Key Words:** cell- and tissue-based therapy ■ induced pluripotent stem cell ■ myocardial infarction  
■ regenerative medicine ■ tissue engineering

Myocardial infarction (MI) secondary to coronary artery disease is a leading cause of morbidity and death throughout the world. Although reperfusion therapies have provided dramatic advances in the treatment of acute MI, a substantial fraction of patients is not able to undergo

successful reperfusion promptly. In patients having large MIs, loss of more than a billion cardiomyocytes can occur, overwhelming the hearts intrinsic reparative capacity. Without further intervention, the damaged myocardium is replaced by fibrous noncontractile tissue (scar), and the resulting left

Original received December 8, 2013; revision received February 24, 2014; accepted March 4, 2014. In February 2014, the average time from submission to first decision for all original research papers submitted to *Circulation Research* was 13.8 days.

From the Department of Medicine (P.A.L., A.N.R., T.J.K.), Molecular and Cellular Pharmacology Program (P.A.L., T.J.K.), and Stem Cell and Regenerative Medicine Center (P.A.L., D.J.H., A.N.R., T.J.K.), Waisman Biomanufacturing at University of Wisconsin, Madison (D.J.H.).

Correspondence to Timothy J. Kamp, MD, PhD, University of Wisconsin, Madison, School of Medicine and Public Health, MC 3248, 600 Highland Ave, Madison, WI 53792. E-mail tj@medicine.wisc.edu

© 2014 American Heart Association, Inc.

*Circulation Research* is available at <http://circres.ahajournals.org>

DOI: 10.1161/CIRCRESAHA.114.300556

## Nonstandard Abbreviations and Acronyms

<b>CPCs</b>	cardiac progenitors cells
<b>EB</b>	embryoid body
<b>ECs</b>	endothelial cells
<b>ESCs</b>	embryonic stem cells
<b>iPSCs</b>	induced pluripotent stem cells
<b>LV</b>	left ventricular
<b>MCBs</b>	Master Cell Banks
<b>MEFs</b>	mouse embryonic fibroblasts
<b>MI</b>	myocardial infarction
<b>MSCs</b>	mesenchymal stem cells
<b>QC</b>	quality control
<b>VSMCs</b>	vascular smooth muscle cells
<b>Wnt</b>	wingless/INT protein

ventricular (LV) dysfunction can initiate a spiral of adverse remodeling progressing to end-stage heart failure. Although pharmacological therapies can blunt the adverse remodeling, the prognosis for these patients is poor with increased risk of death and reduced quality of life. Hence, there is a great need for new approaches to treat patients post-MI, and over the past decade cell therapy has emerged as an appealing avenue to repair the damaged myocardium.

A wide variety of autologous (self) and allogeneic (non-self) cell sources has been tested for post-MI cardiac repair (Table 1). The first cell source studied in detail in both animal models and humans was autologous skeletal myoblasts. Although myoblasts formed stable grafts in the heart, they failed to differentiate into cardiomyocytes and were unable to improve myocardial function.<sup>1</sup> Autologous bone marrow mononuclear cells, which possess a broader differentiation potential than myoblasts, have also been tested in animal models and clinical trials. A range of rodent post-MI studies provided clear evidence for functional benefit resulting from

**Table 1. Cell Sources Investigated for Cardiac Therapy**

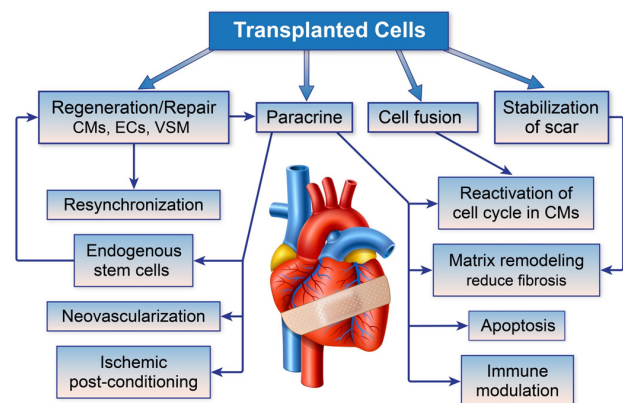
Autologous	Allogeneic
Skeletal myoblasts	Fetal cardiomyocytes
Bone marrow–derived cells	Embryonic stem cells and derivatives
c-kit <sup>+</sup> lin <sup>−</sup>	
Bone marrow mononuclear cells	
Endothelial progenitor cells	Mesenchymal stem cells*†
CD34+	
CD133+	
Cardiac stem/progenitor cells	Parthenogenetic stem cells and derivatives
Side population	
c-kit+	
Cardiosphere derived	
Epicardial progenitors	
Spermatogonial stem cells and derivatives	
Induced pluripotent stem cells and derivatives*	

\*Can be both autologous and allogeneic.

†Can be derived from multiple tissues, including bone marrow and adipose.

transplantation of bone marrow–derived mononuclear cells to the infarcted myocardium; however, the mechanisms of benefit have been debated. In some studies, robust remuscularization of the infarcted myocardium by c-Kit<sup>+</sup>/lin<sup>−</sup> cells isolated from bone marrow was demonstrated,<sup>2</sup> but this finding was contested in other studies, suggesting that bone marrow mononuclear cells do not transdifferentiate into cardiomyocytes.<sup>3,4</sup> Other mechanisms of benefit have also been proposed, including cell fusion, immune-modulation, paracrine effects, and scar stabilization (Figure 1). Despite questions about underlying mechanisms, bone marrow mononuclear cells are being broadly tested in early stage clinical trials with results ranging from a small benefit on cardiac functional parameters to no significant effect.<sup>5,6</sup> Patient-specific cardiac stem cells isolated from the adult heart hold promise.<sup>7,8</sup> Experiments in animal models and more recently in early stage clinical trials have shown encouraging results testing autologous cardiac stem cells and cardiosphere-derived cells.<sup>9,10</sup> However, scalability, senescence, and dysfunction secondary to the underlying pathology are major potential limitations for cardiac stem cells.<sup>11,12</sup> Alternatively, the use of allogeneic mesenchymal stem cells (MSCs) has been investigated. MSCs can be derived from a variety of tissues, including bone marrow and adipose, and these cells can be extensively expanded in culture and exhibit apparent immune privilege. MSCs transplanted post-MI animal hearts have shown benefits, which seem to be primarily paracrine in nature.<sup>13,14</sup> MSCs have also been tested in early phase clinical trials, including MSCs treated with a cardiogenic cocktail, and show signs of functional benefits.<sup>15,16</sup>

Embryonic stem cells (ESCs) provide another allogeneic cell source investigated for post-MI therapy and tested in animal models. ESCs have undoubted potency to generate all cell



**Figure 1. Potential mechanisms of benefit of cell therapy to the post-myocardial infarction (MI) heart.** Transplanted cells exert beneficial effects on the damaged myocardium by multiple potential mechanisms. Regeneration of new myocardium from delivered cells is the most appealing mechanism; however, most preclinical and clinical studies to date have suggested that the beneficial effects of post-MI cell therapy are because of paracrine signaling. Paracrine signaling can reduce apoptosis in surviving cells, promote cell cycle activation for repair, prevent adverse remodeling, activate endogenous stem cells, and induce neovascularization. The optimal cell therapy will likely harness multiple of these potential mechanisms for successful cardiac repair. CM indicates cardiomyocyte; EC, endothelial cell; and VSM, vascular smooth muscle.

types present in the heart and both human<sup>17–19</sup> and mouse<sup>20–25</sup> ESCs and their derivatives have shown functional benefit in various animal MI models. Nevertheless, concerns about immune rejection, safety, and the embryonic source of these cells have delayed clinical applications.<sup>26</sup> In addition, other pluripotent stem cell sources, including spermatogonial stem cells and parthenogenetic stem cells, have been suggested as potential cell sources for cardiac repair,<sup>27,28</sup> but little data exist at this time on these cell sources and particularly with regard to human cells.

The recent discovery of induced pluripotent stem cells (iPSCs) and other advances in reprogramming technologies, such as induced cardiomyocytes, have provided more potential avenues for cardiac repair.<sup>29–32</sup> Although induced cardiomyocytes and direct in vivo reprogramming post-MI hold promise,<sup>33–35</sup> this review will focus on iPSC applications. Because iPSCs are produced by reprogramming somatic cells, such as dermal fibroblasts, they can provide autologous cells for patients, reducing the risk of immune rejection. Another promising feature of iPSCs is that they can be extensively expanded for the production of large quantities of potentially any cell type desired to repair the myocardium. Despite this promise, iPSCs are incompletely understood, so there are several questions associated with this cell source, including concerns about reprogramming effects, immunogenicity, and tumorigenesis. Initial studies have begun to examine the use of iPSCs and their derivatives for cellular therapy to treat a variety of diseases using animal models. The purpose of this review is to describe the progress in using iPSCs and their derivatives for post-MI cardiac repair, highlighting recent advances in iPSC-related technology and preclinical animal models, as well as to discuss the remaining challenges that need to be overcome for successful transition of iPSC technology to the clinic.

### iPSC Technology

In a 2006 landmark study, Takahashi and Yamanaka<sup>29</sup> demonstrated that mouse fibroblasts could be reprogrammed by ectopically expressing 4 key pluripotency factors (Oct-3/4, Sox2, c-Myc, and Klf4) to a state resembling ESCs in morphology and developmental potential and called these cells iPSCs. Similar reprogramming was soon accomplished using human fibroblasts by the Yamanaka et al and independently by Yu et al<sup>32,36</sup> who used a slightly different combination of transcription factors (*OCT4*, *SOX2*, *NANOG*, and *LIN28*). These major discoveries initiated an avalanche of research advancing reprogramming technology and defining the properties and applications of iPSCs. A brief overview highlighting the advances in iPSC technology will be described, but interested readers are referred to other detailed reviews focused on this topic.<sup>37,38</sup>

More cell types were soon demonstrated to be capable of undergoing factor-based reprogramming. In the mouse system, embryonic fibroblasts (MEFs) and tail tip fibroblasts were first reprogrammed, but soon a variety of cell types from all 3 germ layers were successfully reprogrammed. Likewise, iPSC research in the human system has expanded from using dermal or fetal fibroblasts to a range of cell sources, including T cells,<sup>39,40</sup> fat tissue,<sup>41,42</sup> cord blood,<sup>43</sup> amniotic fluid cells,<sup>44</sup> and even renal tubular cells present in urine.<sup>45</sup> Juvenile

human keratinocytes were shown to be 100-fold more efficient and 2-fold faster at generating iPSCs when compared with other human fibroblasts.<sup>46</sup> Also, hematopoietic stem cells were found to be 300× more efficient at iPSC generation when compared with mature T/B cells.<sup>47</sup> These results are in agreement with the mouse system where progenitor cells from various tissues have been more efficiently reprogrammed to iPSCs than terminally differentiated cells, such as fibroblasts. In general, cell types that are closer to inner cell mass cells/ESCs in the developmental hierarchy will be more efficiently reprogrammed to iPSCs.<sup>37</sup>

The iPSCs reprogrammed from different sources share many properties, including pluripotency and self-renewal; however, differences in epigenetic state, mutational burden, and differentiation capacity may ultimately lead to certain preferred cell sources for reprogramming for clinical applications. For example, dermal fibroblasts subjected to UV exposure and other stresses have potentially a greater mutation burden than more protected blood stem and progenitor cell pools. Alternatively, cell types that require less manipulation for reprogramming to pluripotency may be less likely to have adverse effects from reprogramming. One such cell type is the skeletal myoblasts, which endogenously express Klf4, Sox2, and c-Myc, and hence induced expression of Oct4 alone can reprogram them to pluripotency.<sup>48</sup> Some investigations have also proposed biased differentiation potential of the iPSC line based on the tissue of origin because of epigenetic memory.<sup>49</sup> The relative importance of these and other differences between cell sources regarding clinical applications using iPSCs will require further investigation.

The methods to reprogram somatic cells are also rapidly advancing. Retroviral and lentiviral delivery of reprogramming factors were first used with success. However, limitations with these integrating vectors are well known, including insertional mutagenesis (activating or inactivating an endogenous gene) and persistent expression of the transgene. These effects could affect the functional properties of iPSCs and their progeny, and they are of particular concern for clinical applications given the observed insertional mutagenesis-induced cancers in some early gene therapy trials. Recently, sendai virus-based vectors have been used in iPSC reprogramming.<sup>50</sup> Sendai viruses, unlike retroviruses, do not integrate in host genome and are diluted out of reprogrammed cells over passages. Alternative nonviral/nonintegrating approaches have also been used, including transfecting with simple plasmids,<sup>51</sup> episomal vectors,<sup>36</sup> and mini-circle-based vectors<sup>52</sup>; however, these techniques were inefficient when compared with virus-based vectors. PiggyBac transposon-based vectors have also been used that have higher reprogramming efficiency and can be excised from the genome after achieving reprogramming.<sup>53,54</sup> Nevertheless, DNA-based strategies still carry some risk of insertional mutagenesis and genomic recombination, which needs to be considered. Therefore, recombinant proteins were used for reprogramming mouse fibroblasts.<sup>55</sup> This method, although not DNA based, was slow and inefficient. Also, generating recombinant proteins in vitro is a major task. A more recent advance is the use of modified mRNA-based transcription factor delivery as an alternative for reprogramming.<sup>56</sup> However, non-viral-based reprogramming

strategies have, in general, been less efficient when compared with virus based. A recent study has revealed that activation of innate immune response (as triggered by viral infection) leads to epigenetic changes that favor reprogramming.<sup>57</sup> This discovery could potentially be applied to nonintegrating approaches to increase reprogramming efficiency further and to get safer iPSC for transplant. Finally, epigenetic modifiers, such as histone deacetylase inhibitors and DNA demethylating agents, have been used to increase reprogramming efficiency in conjunction with the abovementioned strategies.<sup>58</sup>

In conclusion, there has been exponential growth in the technologies to produce iPSCs, which has outpaced the ability to investigate thoroughly and benchmark the properties of resulting iPSCs. Nevertheless, iPSCs are already proving useful in drug testing and disease modeling. To date, a variety of patient-specific iPSC lines have been generated for hematopoietic, hepatic, neurological, and cardiovascular diseases, demonstrating the use of iPSC technology for studying disease mechanisms *in vitro*. iPSC-derived cardiomyocytes and neurons along with other cells types are increasingly being used in the pharmaceutical industry for drug discovery and toxicity assays.<sup>59,60</sup> Growing experience with these nonclinical applications of iPSCs has helped lead to the general consensus that nonintegrating strategies to produce footprint-free iPSCs should be given the highest priority for further characterization and preclinical studies.

### **Differentiation and Purification of Cardiac Lineage Cells From iPSCs**

ESCs and iPSCs have an unquestionable cardiac differentiation potential, like the pluripotent cells in the inner cell mass of the blastocyst that undergo a series of sequential differentiation steps, including formation of mesodermal cells that further develop to heart field-specific progenitors that ultimately give rise to the heart. This complex process is orchestrated by dynamic growth factor gradients specific for particular stages of development and changing extracellular matrix composition as reviewed well by others.<sup>61,62</sup> Multiple cell types differentiate and precisely organize to form the functioning heart, including cardiomyocytes, endothelial cells (ECs), vascular smooth muscle cells (VSMCs), and fibroblasts. Initial differentiation protocols used aggregates of ESCs, which in the presence of serum can spontaneously differentiate into embryoid bodies (EBs) containing poorly organized derivatives of the 3 primary germ layers including in some EBs contracting cardiomyocytes. Alternatively, taking advantage of insights from cardiac development, coculturing undifferentiated ESCs on a feeder layer of visceral endoderm-like cells was used to induce cardiogenesis.<sup>63</sup> However, these initial approaches showed significant variability and were relatively inefficient at generating cardiomyocytes. More recently, differentiation protocols aimed at generating cardiomyocytes have evolved to use defined conditions with timed applications of specific growth factors known to be important in cardiac development (eg, activin A, bone morphogenetic protein 4, basic fibroblast growth factor, wingless/INT proteins, and in some cases manipulations of extracellular matrix as reviewed in detail elsewhere).<sup>64</sup> The resulting yield and purity of cardiomyocytes

from these protocols have improved dramatically such that it is relatively straightforward to obtain millions and with scale-up billions of cardiomyocytes at purities of 70% or better. In addition, small molecules have been used to manipulate wingless/INT protein signaling biphasically during differentiation to produce high yields and purities of cardiomyocytes.<sup>65</sup> Although much of the research on differentiation protocols was done with human ESCs, these protocols have also been applied to iPSCs successfully. The advances in cardiomyocyte differentiation protocols represent an enabling advance in the technology for producing human heart cells for potential clinical applications.<sup>64</sup>

Like protocols for generating cardiomyocytes from ESCs and iPSCs, continually improving protocols for the differentiation to VSMCs and ECs have been evolving. Initial approaches used EBs to generate ECs and VSMCs, but subsequent studies have used feeder cells secreting key signaling molecules or more directed differentiation approaches. In directed differentiation protocols, growth factors to promote differentiation to VSMCs include platelet-derived growth factor-BB and transforming growth factor- $\beta$ 1.<sup>66,67</sup> In the case of differentiation to ECs, bone morphogenetic protein 4 and vascular endothelial growth factor-A have been commonly used.<sup>66</sup> Vascular progenitor cells have been isolated from cultures based on CD34 expression that can give rise to both ECs and VSMCs.<sup>66,68,69</sup> For ECs, purification of the cells has been accomplished using anti-CD31 or anti-vWF with sorting to give relatively pure populations of ECs. Differentiation of other clinically relevant cells from iPSCs and ESCs has been described, including cardiac progenitors and MSCs.<sup>70–73</sup> Thus, ESCs/iPSCs can be differentiated into multiple cell types, which are potentially useful for cardiac cell therapy.

Although the progress with differentiation protocols has been striking, a closer examination of the resulting cell populations reveals that significant cellular heterogeneity remains, which could adversely affect clinical outcomes. In the case of cardiomyocyte differentiation, even if a protocol generates predominately cardiomyocytes, there are different types of cardiomyocytes present, which exhibit distinct functional properties including ventricular-like, atrial-like, and nodal-like cardiomyocytes.<sup>74</sup> If the purpose of a cell product is to repair the ventricular myocardium, then ventricular-like cells would likely be the desired cardiomyocyte population. In addition, noncardiomyocyte cells are present typically, including VSMCs, ECs, fibroblasts, and potentially other undefined cell types. Furthermore, the cells can exhibit variable functional maturity and typically are embryonic in phenotype. Similar concerns exist for other lineage differentiation protocols, such as EC preparations, which exhibit a mixture of arterial, venous, and lymphatic ECs.<sup>75</sup> Several approaches are being advanced to address such heterogeneity, such as cell sorting, using cell surface markers. In the case of ECs, several well-defined cell surface markers are known, such as cluster of differentiation 31 (CD31) or von Willebrand factor (vWF), but for cardiomyocytes, the cell surface markers are only starting to be defined and used, such as vascular cell adhesion protein 1 (VCAM) and signal-regulatory protein alpha (SIRPA).<sup>71,76</sup> Genetic selection strategies in which cells



are genetically modified with a cell lineage-specific promoter driving the expression of a fluorescent protein or an antibiotic resistance gene can allow selection of the desired cell type.<sup>77,78</sup> The disadvantage of this approach is that it requires genetic manipulation of the cells, which can be time-consuming and create adverse consequences, such as insertional mutagenesis. Recently, differences in the metabolic properties between cardiomyocytes and noncardiomyocytes have been exploited to devise a metabolic selection strategy, which involves substituting lactate for glucose in culture medium to obtain cardiomyocytes of >99% purity.<sup>79</sup> Ultimately, refining differentiation protocols will produce improved preparations for therapy. Whether pure cell lineage preparations are ideal or a mixture of cells is best will require additional investigation.

### Animal Studies Using iPSC and iPSC Derivatives for Post-MI Repair

A growing number of studies have tested the ability of iPSCs and differentiated cells derived from iPSCs to repair the post-MI heart (Table 2). Most of the studies have been in small animal models, but large animal models are also starting to be used. The cell preparations being tested have included undifferentiated iPSCs, iPSC-derived cardiac progenitors, cardiomyocytes, VSMCs, and ECs (Figure 2). These cell preparations have been tested alone or in combination sometimes using tissue engineering approaches.

Initial studies of iPSCs for cardiac repair tested undifferentiated iPSCs in animal models. The first study using iPSCs for post-MI repair was undertaken by Nelson et al.<sup>80</sup> They used human Oct4, Sox2, Klf4, and c-Myc (Yamanaka factors) incorporated in lentiviral delivery vectors to reprogram mouse embryonic fibroblasts (MEFs) to miPSCs. Undifferentiated miPSCs were injected intramyocardially in either immunocompetent or immunodeficient mice after left coronary artery ligation. Four weeks after transplantation, the immunocompetent mice showed significant improvement in ventricular function and reduced pathological remodeling because iPSC transplantation. Histological analysis showed iPSC-derived cardiomyocytes, VSMCs, and ECs within damaged myocardium, confirming the capacity of the injected cells to differentiate into the 3 primary cardiac lineages *in vivo* without evidence of tumor formation. In contrast, the majority of the immunodeficient mice developed tumors 2 weeks after iPSC injection and their cardiac function was progressively compromised due to tumor burden. Similarly, the Singla group transplanted undifferentiated miPSCs derived from H9c2 cardiomyoblast cells (isolated from embryonic ventricular tissue) into the peri-infarct region of immunocompetent mice and reported improvement in LV function, as well as reduced apoptosis because of engraftment of iPSC progeny 2 weeks after transplant.<sup>81</sup> No obvious teratomas were reported in the short time span of these studies. The above reports suggest that transplanting low numbers of pluripotent cells into immunocompetent post-MI mouse hearts results in cardiac-specific differentiation of iPSCs and cardiac repair. However, Ahmed et al.<sup>82</sup> demonstrated that iPSCs derived from VSMCs, when injected after MI, were tumorigenic in ~40% of the mice. Others using rat iPSCs in an immunocompetent, allogeneic rat model reported tumor incidences (both intra- and extracardiac sites) that were

independent of cell dose, transplant duration, and presence or absence of MI.<sup>83</sup> These reports challenged the hypothesis that cardiac microenvironment (native or postinfarct) is sufficient to direct pluripotent cells exclusively toward cardiac lineages. In general, the field has accepted that undifferentiated pluripotent cells (ES or iPS) are undesirable for clinical applications because of their tumorigenic potential; nonetheless, the above studies provided proof-of-principle that iPSCs hold promise for post-MI cell therapy.

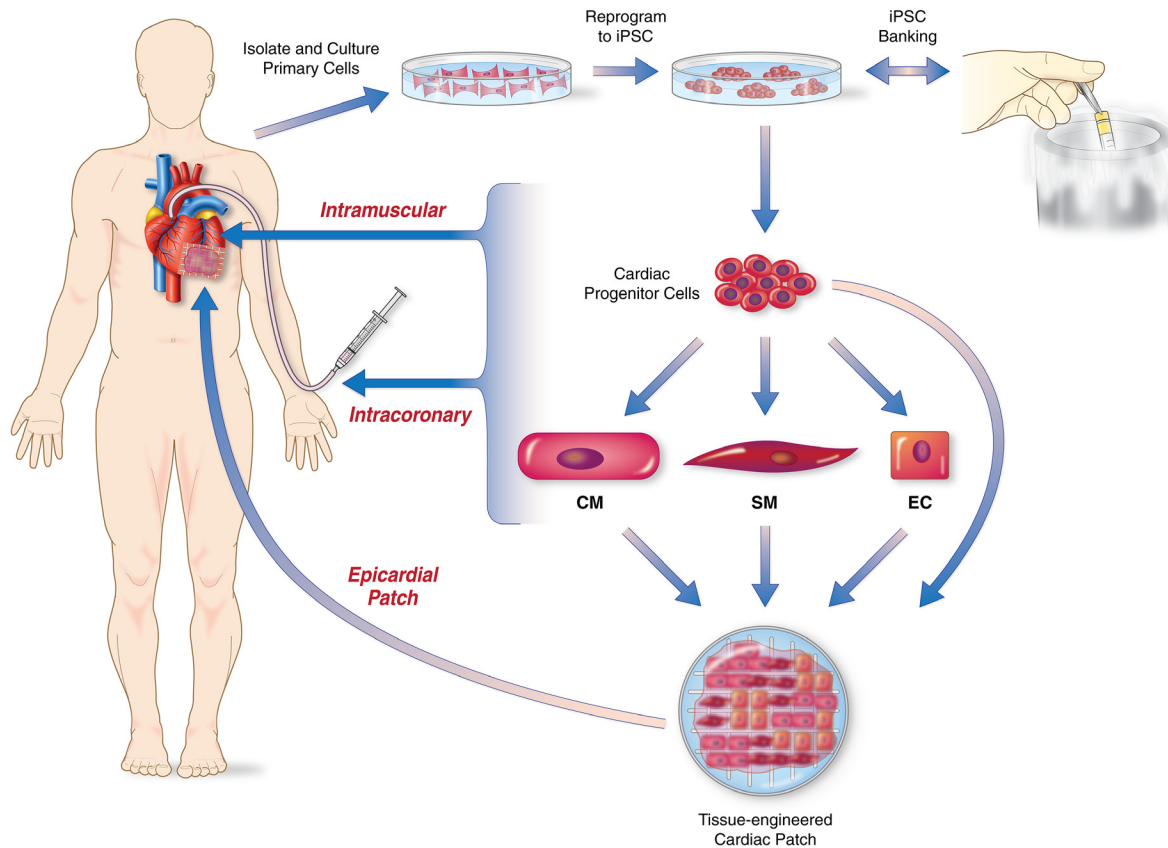
Ongoing studies have focused on testing differentiated iPSC derivatives for post-MI repair. One strategy to obtain differentiated iPSC derivatives is to differentiate the iPSCs in EBs and isolate the contracting regions. The contracting cell aggregates can be enzymatically treated to provide singularized cardiomyocytes, cardiac progenitors, and likely other cell types. Using this cell isolation approach, Pasha et al.<sup>48</sup> transplanted cells into infarcted areas of immunocompetent mice immediately after left anterior descending (LAD) coronary artery ligation. Four weeks after transplantation, reduced pathological remodeling and improved ventricular contractility were observed without tumor formation. The same group has also used microdissected beating aggregates from retrovirally reprogrammed skeletal myoblasts-iPSCs<sup>84</sup> and bone marrow MSC-derived iPSCs<sup>85</sup> for post-MI injection and reported positive effects on cardiac function. The positive effects were partly attributed to paracrine signaling. Although the authors refer to cells isolated from microdissected EBs as cardiac progenitor cells/cardiomyocytes, it should be noted that spontaneous EB differentiation results in a mixture of contracting cardiomyocytes, differentiating cardiac progenitor cells, VSMCs, ECs, and potentially cell types of other lineages. Hence, the beneficial effects on cardiac function reported in these studies are complex because it is difficult to tease out the exact contribution of the various undefined cell types that were injected after MI. However, it is important to note that mice injected with microdissected beating clusters in these studies did not form teratomas, unlike the undifferentiated iPSCs. These experiments demonstrate the potential use of using predifferentiated iPSC-derived cell types for post-MI repair.

To provide more defined, differentiated cell populations for transplantation, Dai et al.<sup>86</sup> engineered a transgenic mouse iPS cell line that expressed green fluorescent protein (GFP) under the control of cardiac-specific NCX1 promoter to purify cardiomyocytes from differentiating EBs. In this study, a tissue patch was generated for application to the post-MI heart by seeding purified iPSC-cardiomyocytes, CD31-selected iPSC-ECs, and mouse embryonic fibroblasts (MEFs) on rat peritoneum patches (Tri-patch). Inclusion of MEFs dramatically improved the formation of organized blood vessels throughout the patch. The Tri-patches were placed over the infarcted myocardium 1 week after coronary ligation. Four weeks after the patch implant, the mice that received the Tri-patch showed a significant reduction in LV fibrosis when compared with MEFs-only patch. This study did not perform comparative analysis between the functional benefits from cardiomyocyte-only or EC-only patches when compared with Tri-patch. Also, there was no information comparing direct cell injection versus Tri-patch

**Table 2. Preclinical Studies of Post-MI Cell Therapy Using iPSCs and Derivatives**

References	Cell Source for Reprogramming	Reprogramming Method	Cell Type Transplanted	Delivery Method	Animal Model	Duration of Study	Summary of Results
Nelson et al <sup>80</sup>	MEFs	Lentiviral-human (KOSM)	iPSCs	Intramyocardial injection	Mouse	4 wk	↑ Ventricular function, ↓ pathological remodeling. Engraftment and differentiation into CMs, SMs, ECs. No teratomas in immunocompetent mice. Teratomas in immunodeficient mice
Singla et al <sup>81</sup>	Mouse H9c2 cardiomyoblasts	Plasmid-mouse (KOSM)	iPSCs	Intramyocardial injection	Mouse	2 wk	↑ Ventricular function, ↓ apoptosis. No teratomas in immunocompetent mice
Templin et al <sup>83</sup>	Human cord blood	Lentiviral-human (NOLS)	iPSCs	Intramyocardial injection	Pig	12–15 wk	iPSCs coinjected with human MSC survived and differentiated into endothelial cells. iPSCs injected alone failed to survive. Pigs were immunosuppressed
Ahmed et al. <sup>82</sup>	Mouse SMs	Retrovirus-mouse (KOSM)	iPSCs	Intramyocardial injection	Mouse	4 wk	Tumorigenic in 40% of immunocompetent mice
Zhang et al <sup>83</sup>	Rat bone marrow	Lentiviral-human (KOSM)	iPSCs	Intramyocardial injection	Rat	2–6 wk	Tumorigenic independent of cell dose, duration, and presence/absence of MI
Pasha et al <sup>48</sup>	Mouse SMs	DNMT-RG108	Day5 EB-beating aggregates	Intramyocardial injection	Mouse	4 wk	↓ Fibrosis, ↑ heart function. No teratomas in immunocompetent mice
Ahmed et al <sup>84</sup>	Mouse SMs	Retrovirus-mouse (KOSM)	Day10 EB-beating aggregates	Intramyocardial injection	Mouse	4 wk	↓ Infarct size, ↑ cardiac function. No tumorigenesis
Buccini et al <sup>85</sup>	Mouse bone marrow MSCs	Retrovirus-mouse (KOSM)	Day10 EB-beating aggregates	Intramyocardial injection	Mouse	4 wk	↓ Infarct size, ↑ global cardiac function partly because of paracrine effects. No tumorigenesis
Dai et al <sup>86</sup>	MEFs	Plasmid-mouse (KOSM)	Day14 EB-NCX1+ CMs+ ECs+MEFs	Rat peritoneum patch	Mouse	4 wk	↓ Fibrosis, engraftment, improved LV function
Kawamura et al <sup>86</sup>	Human dermal fibroblasts	Retrovirus-human (KOSM)	Day 25 CMs + human dermal fibroblasts	Scaffold-free cell patch	Pig	8 wk	Few hiPSC-CMs were retained at infarct site 8 wk after transplant. ↑ cardiac performance, ↓ ventricular remodeling attributed majorly to paracrine signaling. No teratomas
Mauritz et al <sup>90</sup>	MEFs	Retrovirus-mouse (KOSM)	Day5 EB-Flk1+ CPCs	Intramyocardial injection	Mouse	2 wk	↑ Graft size and LV wall thickening in mice that received Flk1+ CPCs when compared with Flk1- cells
Xiong et al <sup>97</sup>	Human dermal fibroblasts	Lentivirus-human (KOSM)	iPSC-derived ECs+VSMSs	Epicardial fibrin patch	Pig	4 wk	Mobilization of endogenous progenitors, ↑ LV functions, ↓ scar size, neovascularization, ↑ metabolism of borderzone myocardium in cell-treated immunosuppressed pigs when compared with controls

CMs indicates cardiomyocytes; CPC, cardiac progenitors cells; EB, embryoid body; ECs, endothelial cells; hiPSCs, human induced pluripotent stem cell; KOSM, Klf4+Oct4+Sox2+cMyc; LV, left ventricular; MEFs, mouse embryonic fibroblasts; MI, myocardial infarction; NOLS, Nanog+Oct4+Lin28+Sox2; and SMs, skeletal myoblast.



**Figure 2. Cardiac cell therapy strategies using induced pluripotent stem cells (iPSCs) and derivatives.** Patient-specific primary cells are isolated and cultured *in vitro* from a suitable cell source, such as blood or skin. These cells are reprogrammed to iPSCs using nonintegrating strategies under Current Good Manufacturing Practice conditions. The resulting iPSCs are rigorously tested for pluripotency, genetic/epigenetic abnormalities, and safety (Tables 3 and 4). The iPSC clones that pass test criteria are banked for later use. Alternatively, allogeneic iPSCs can be used from a haplotype-matched iPSC bank. The iPSCs are then differentiated into the desired cardiac lineage cells: cardiac progenitors, cardiomyocytes (CMs), smooth muscle (SM) cells, or endothelial cells (ECs). The desired cell lineage or combination of cell lineages is transplanted into damaged heart via intracoronary/intramuscular injection or epicardially by tissue engineered cardiac patches. Ongoing studies will define the optimal cell preparations and associated delivery strategies for repair of the post-myocardial infarction heart.

application for post-MI therapy. The use of MEFs in this study is another limitation because this cell source is heterogeneous, derived broadly from mouse embryos, and comparable populations for human clinical applications are not available. Nevertheless, this study was the first study to examine cardiac tissue engineering with iPSC therapy.

It is a matter of debate whether terminally differentiated cell types, such as cardiomyocytes, VSMCs, or ECs, when compared with multipotent, yet tissue-committed, cardiac progenitors cells (CPCs) will be more suitable for cardiac repair. CPCs when compared with differentiated cardiomyocytes are scalable and can be propagated to generate large number of cells as would be required for post-MI cell therapy. CPCs are multipotent and can differentiate into VSMCs and ECs along with cardiomyocytes. These cell types are equally important for normal function of the heart. Also, CPCs may have superior mechanical and electric coupling capability when compared with cardiomyocytes and other differentiated cell types when delivered to the injured myocardium.<sup>87–89</sup> The first proof-of-principle study using iPSC-derived defined CPCs in post-MI cell therapy was published by Mauritz et al.<sup>90</sup> They

retrovirally reprogrammed MEFs into iPSCs and isolated Flk1<sup>+</sup>/Oct4<sup>+</sup> CPCs from day 5 differentiated EBs via fluorescence activated cell sorting. The Flk1<sup>+</sup> and Flk1<sup>−</sup> cells were then injected into mouse LV walls after LAD ligation, and heart function was monitored for 2 weeks. Reduced cardiac remodeling and improved function was observed in both groups when compared with PBS controls. Mice that received Flk1<sup>+</sup> cells had significantly larger grafts and increased LV wall thickening. The Keller group was the first to identify Flk1 (KDR in humans) as a marker for CPCs and is the most extensively characterized CPC surface marker to date.<sup>91,92</sup> Although Flk1 also marks hematopoietic stem cells at an earlier stage in development, it has been well documented that cardiac lineage cells are enriched in Flk1 expressing cells from day 4 onward in mouse EB differentiation. This report puts forth a convincing argument that iPSC-derived CPCs hold promise for future transplantation studies.

For iPSC technology to reach clinical applications, preclinical testing is necessary with human iPSCs (hiPSCs) and in large animal models, such as pigs, canines, sheep, or nonhuman primates. The tissue volume and number of functioning

cardiomyocytes in these large animals are much more representative of human hearts. Furthermore, clinically relevant imaging and delivery techniques can be used in these large animal models. Templin et al<sup>93</sup> published an initial study reporting the use of human iPSCs in a pig MI model. They engineered a transgenic human iPSC line expressing sodium iodide symporter to enable long-term tracking of transplanted cells via single photon emission computed topographical imaging. Ten days after inducing an MI in pigs, hiPSCs/hiPSCs+human MSCs were introduced by an intramyocardial injection and imaged for  $\leq 15$  weeks ( $^{123}\text{I}$  was infused by intracoronary injection before imaging).  $^{123}\text{I}$  signal was detected at the injection site for hiPSC+hMSC group 12 to 15 weeks after injection, where the hiPSC differentiated into ECs. On the contrary, no signal was detected in the hiPSC-only group. This indicates that hMSC coinjection was critical for the long-term survival and engraftment of transplanted hiPSCs. MSCs have been reported to secrete antiapoptotic and immunomodulatory factors that facilitate post-MI repair.<sup>94,95</sup> No teratomas were detected during the course this study, even though the pigs were treated with immunosuppressant drugs. In another recent large animal study, Kawamura et al<sup>96</sup> developed a scaffoldless hiPSC-derived cardiomyocyte cell sheet for application in a pig MI model. The iPSCs were reprogrammed from human dermal fibroblast via retroviral infection of Yamanaka factors and differentiated into high purity cardiomyocytes by modulating the wntless/INT protein pathway. A small amount of fibroblasts and ECs were also present in the differentiated cultures. Cardiomyocytes were then seeded onto UpCell dishes (CellSeed, Tokyo, Japan) along with human dermal fibroblasts to form scaffold-free hiPSC-cardiomyocytes cell sheet. UpCell dishes are thermosensitive and cause cells to detach spontaneously and form a sheet when incubated at room temperature. These cell sheets were then transplanted over the infarcted myocardium and cardiac functioning, and cardiomyocyte engraftment was monitored after 8 weeks. Animals transplanted with hiPSC-cardiomyocytes sheets showed significant improvement in cardiac function and had reduced fibrosis and increased capillary density around the infarct region when compared with controls. The positive effects were mainly attributed to paracrine signaling. Few of the transplanted cardiomyocytes engrafted into the host MI site. The majority of the cells were lost, which may have been because of insufficient immunosuppression. One study tested the effect of transplanting hiPSC-derived ECs combined with hiPSC-derived smooth muscle cells in an immunosuppressed pig ischemia/reperfusion model.<sup>97</sup> The iPSC-ECs and iPSC-VSMCs were delivered via an epicardial fibrin patch over the infarct bed. This study demonstrated improvements in LV function with reduced scar size in the cell-treated group relative to placebo group based on MRI. In addition, the function and metabolism of borderzone myocardium were improved by cell therapy. Histology provided evidence for neovascularization of the borderzone with some smooth muscle cells and ECs being of human origins in the capillary network. These important studies provided the first test of hiPSC derivatives in a pre-clinical large animal model and provided encouraging results. Future studies testing other iPSCs-derived cell lineages, such

as cardiac progenitors or cardiomyocytes in these large animal models, are anticipated and may provide even better repair. However, this is just the beginning of large animal studies, and future work will need to examine dose optimization, delivery methods, and long-term safety end points.

As in studies with other cell sources post-MI, multiple underlying mechanisms of benefit have been described after transplantation of iPSCs and iPSC derivatives (Figure 1). Remuscularization of the heart has been the focus, but typically the extent of transplanted cell engraftment and survival has been relatively sparse, suggesting the effect exceeds that expected for small grafts. A recent study transplanting porcine iPSC-ECs into a mouse model provided compelling evidence that the improvement in myocardial function largely occurred by a paracrine mechanism.<sup>98</sup> In the future, the cellular preparation(s) prepared from iPSCs can be optimized to harness the most potent mechanisms for repair.

### Transitioning to Clinical Trials and Current Good Manufacturing Practice Processing of iPSC Products

The path from the research laboratory to clinical trials for iPSC-based therapeutics shares similarities to other cell therapeutics in many respects, but there are also unique aspects. The shared goals for cell products include developing a reproducible manufacturing process, eliminating or minimizing the use of raw materials that introduce risk, establishing suitable quality control (QC) test methods, and establishing the procedures and documentation required to produce the cell therapeutic according to current Good Manufacturing Practice and current Good Tissue Practice guidelines. Compliance with the current Good Manufacturing Practice regulations is a continuum of increasing compliance from the initial production of material for phase 1/2a clinical trials through to final launch of the commercial product. In addition, relatively unique considerations arise about the use of iPSC products in cell therapy, including the quality, robustness, and safety of the reprogramming process, distinct approaches for autologous when compared with allogeneic applications, and a reproducible differentiation process to produce highly defined cellular products free of undifferentiated pluripotent cells.

### Considerations for Autologous Versus Allogeneic

One of the key considerations for developing an iPSC therapeutic is whether cell products will be autologous or allogeneic. An autologous therapeutic obviously provides a major benefit from the standpoint of addressing the key issue of rejection of the cell transplant. However, the choice of an autologous therapeutic also comes with several potential challenges from a manufacturing and regulatory perspective. First, a manufacturing process for an autologous therapeutic must be able to handle the wide range of patient-to-patient variability. Differences in patient age, disease condition, and genetic/epigenetic background can all potentially affect the consistency of the quality and safety of the final cell therapeutics. Extensive process development and process validation studies may be required to demonstrate the ability of the process to handle the variability in the starting cell source. It is also worth noting that an autologous therapeutic may pose an increased risk of tumor



formation because the patient would not be expected to launch an immune response against the autologous graft.

The alternative choice of an allogeneic cell therapeutic allows well-characterized cell banks to be established for each therapeutic. This is a key consideration in applications where genetically modified cells are used to deliver therapeutic proteins. The issue of rejection is obviously a key consideration for allogeneic cell therapeutics. However, the ability to generate human leukocyte antigen diverse banks offers one potential solution to this problem. Requirements for the use of immunosuppression may be minimized or potentially eliminated depending on the tissue that is being replaced and the required degree of human leukocyte antigen matching. For example, it has been suggested that iPSCs derived from 150 unique human leukocyte antigen-homozygous donors could cover 90% of the Japanese population.<sup>99</sup> Because of greater genetic diversity, many more unique donors would be required to cover the American population.

### Early Stage Process and Assay Development

One of the biggest challenges in harnessing the potential of iPSCs is the inherent variability in the cell manufacturing process. This variability can be caused by variability in the quality of the starting cell source, differences in the quality of critical raw materials, differences in growth and differentiation protocols, and differences in testing methods. Identifying and controlling the variability in the process and demonstrating that it does not introduce unacceptable risk is an important requirement for developing a current Good Manufacturing Practice compliant manufacturing process. For example, a key requirement for cell therapeutics is that they be produced in a manner that prevents the introduction of microbial or fungal contaminants at any step in the process. Standardized, robust methods for growing cells, evaluating critical raw materials (eg, medium and growth factors), differentiating cells, and performing QC testing are needed early in the development program.

Development, qualification, and validation of QC assays are another key aspect of development that must be addressed early. QC assays are typically developed and qualified in parallel with the manufacturing process. Key QC assays should be developed and qualified before initiating process qualification studies so that these assay are in place to support analysis of product from the qualification runs. Qualification involves demonstrating assay capabilities, and that the assay is suitable for the intended application. Establishing a reference standard for the cell therapeutic is also important early in the development because this will allow variability in the assay to be tracked. Potency assays represent one of larger challenges for assay development.

### Material for Preclinical Testing and Human Clinical Trials

Once the process and assay development stage of the project is completed, material is typically produced for the preclinical animal studies to support the Investigation New Drug application that will be filed with the Food and Drug Administration before human clinical trials. A key consideration in producing the cells for animal studies is to ensure that the final cell product used in the animal studies is representative of the cell therapeutic that will be used in the human clinical trial. If

possible, identical cell banks, raw materials, process, and QC testing should be used to produce the cells for animal studies. Again, variability in the starting cell source for autologous cell therapeutics may represent a significant challenge in determining suitable cells for evaluation in preclinical animal studies.

The preclinical animal studies must address areas of concern about the safety of the transplanted cell product. The concerns include the effect of off-target cells and the potential for teratoma formation because of residual undifferentiated cells or the generation of karyotypically abnormal cells. It is, therefore, critical that aspects of the process that could affect the quality of the cells with respect to attributes, such as residual undifferentiated cells included in starting cell material, are identical between cells produced for animal studies and human clinical trials. A dialogue should be established with the Food and Drug Administration to ensure that any intended process changes that are anticipated in moving from animal studies to clinical production do not represent significant changes.

### iPSC Bank Production

For allogeneic cell therapeutics, establishing a bank of the starting cell material is usually done in the early stages of development. Typically a single Master Cell Bank (MCB) or a 2-tiered system with a Working Cell Bank is produced and tested to insure that the starting material for the manufacturing process is consistent. For autologous iPSC therapeutics, establishing a large iPSC bank is not necessary; however, establishing a small bank of low passage iPSCs after the reprogramming step will allow variability from the reprogramming step to be identified and potentially minimized. Establishing an intermediate bank with some level of QC testing will help to insure that a more consistent iPSC will enter the differentiation steps of the manufacturing process. Moreover, the existence of the intermediate bank will allow production of additional cells if the differentiation stage of the process is not successful or additional cells are needed for treatment.

The iPSC MCBs are produced starting with iPSC clones from the reprogramming process. Several iPSC colonies are typically selected and screened to ensure that they meet specifications, such as no bacterial/fungal/mycoplasma contamination, acceptable growth characteristics, normal karyotype, and appropriate iPSC marker expression. An iPSC clone that meets these specifications is selected and moved into production and expanded to create the MCB using a manufacturing process that is clearly defined in manufacturing procedures. The manufacturing process should minimize the use of animal-derived raw materials wherever possible, and complete traceability should be maintained for all materials used in MCB production. Cell bank production is performed according to established manufacturing standard operating procedures (SOPs). Methods have previously been described for the expansion of pluripotent stem cells using completely defined, xeno-free cell culture medium and attachment matrix.<sup>100,101</sup> All aspects of the process are documented from starting cell source and all materials used in reprogramming and production through to the final harvest, cryopreservation, and QC testing. Cell Bank testing for adventitious agents is a critical part of developing

cell lines for use in clinical production. Typical testing that is performed on an iPSC MCB is outlined in Table 3.

### iPSC Differentiation to the Final Cell Product

The differentiation stage of the manufacturing process should be initiated using iPSCs from a cell bank or culture that has been subjected to the QC testing discussed above. This will ensure that cells entering the differentiation process meet key criteria to help minimize variability in the differentiation process. The differentiation process should be developed and optimized to address 4 main issues: minimize undefined or animal-derived raw materials, ensure that the process is sufficiently robust to handle variability in the starting iPSCs, ensure that the process is scalable to meet future projected demands, and minimize off-target cell types and residual undifferentiated cells.

QC testing of the final cell therapy should be designed to accommodate the product format and preparation procedures. Abbreviated QC testing may be required for releasing products that are prepared fresh for each administration. A summary of typical QC testing for an iPSC therapeutic is provided in Table 4. Some of these tests may be performed on intermediate cells, whereas others may have a rapid method that is performed before the release that is followed by a more rigorous method. For example, in cases where fresh cells are administered to patients, there is not sufficient time to perform the standard sterility test that requires a 14-day incubation period. In this case, the Food and Drug Administration typically asks for a rapid test method (eg, Gram stain) to detect contamination with a follow-up full sterility test. A plan must be in place to address the potential scenario of a failed sterility test of a product that has already been administered to a patient. Other critical assays, such as residual undifferentiated cells, will be more challenging to address in this situation and may need to be performed on an intermediate sample so that results are available before the release of the cell therapeutic to the clinic.

### Clinically Relevant Cell Delivery and Tracking

It is appealing to consider that iPSCs or their derivatives may home, engraft, mechanically and functionally couple with the infarcted myocardium, regenerate and improve cardiac contractility. However, the myocardium is an extremely complex and dynamic tissue with cells aligned and interconnected in a structured extracellular matrix that is highly vascularized. Further complicating this area of investigation is that methods used to deliver cells may be just as important therapeutically as the intrinsic regenerative potential of the cells themselves. Despite numerous cardiac cell therapy trials worldwide, variables, such as the cell delivery method, infusion rates, timing, geographic distribution and dose, continue to remain poorly defined.

A variety of cardiac cell delivery methods has been described and can be broadly categorized as (1) intravascular infusion (ie. peripheral vein infusion, direct antegrade coronary artery infusion, direct retrograde coronary sinus infusion), (2) intramuscular injection (epicardial injection, transendocardial needle injection), and (3) cellular scaffolds that are applied to the epicardial surface.<sup>102,103</sup> Each delivery method has both advantages and disadvantages. Peripheral vein infusion is simple, inexpensive, and noninvasive, but cell retention in the heart is extremely low. Intravenous infusion relies on intact homing and a large proportion of cells trap in the lung vasculature.<sup>104</sup> Direct intracoronary and retrograde coronary sinus infusion can be performed minimally invasively using available catheters, but these approaches have the problem of poor cell retention and the need for patent vessels supplying the target myocardium. Large cells, such as ESCs and iPSCs, are not well suited to intravascular infusion because vascular plugging can occur and the ischemic zone may be extended. Epicardial injection through a thoracotomy incision offers improved cell retention and is not dependent on intact vascular conduits; however, this approach is invasive and is perhaps not clinically practical for the recent post-MI patient. Small incision, thoracoscopic delivery is more appealing but injecting

**Table 3. Proposed Testing for iPSC Master Cell Banks**

Test	Description
Identity	STR testing
Viable Cell Count	Trypan blue dye exclusion
Bacterial/fungal contamination	Testing for bacterial and fungal contamination according to 21 CFR 610.12
Mycoplasma contamination	Direct culture in broth and agar, indirect test using indicator culture/DNA stain. Assay conditions meet the FDA's PTC requirements
Karyotype	G-band on 20 metaphase spreads
Cell marker expression	Flow cytometry for human ES cell marker expression (eg, Oct-3/4, SSEA-1/3/4, TRA-1-60, TRA-1-81)
Human pathogen testing	Performed on donor, including HIV 1, HIV 2, HBV, HCV, and <i>Treponema pallidum</i> , and for leukocyte-rich tissues HTLV-I/II and CMV
In vitro adventitious agent testing	ICH cell line testing on 3 cell lines – MRC5, Vero, NIH 3T3/Hs68
Additional pathogen testing	Additional testing performed based on risks introduced by reprogramming vectors and materials used in the manufacturing process
Residual reprogramming vector	PCR or Southern blot to detect and map residual reprogramming vector

CFR indicates code of federal regulation; CMV, cytomegalovirus; ES, embryonic stem; FDA, Food and Drug Administration; HBV, Hepatitis B virus; HCV, Hepatitis C virus; HTLV, human T-lymphotropic virus; ICH, international conference on harmonisation; PCR, polymerase chain reaction; NIH, National Institutes of Health; PTC, points to consider; and STR, short tandem repeat.

**Table 4. Quality Control Testing Plan of the Final Cell Product**

Attribute	Method	Specification
Before and after thaw	Trypan blue or other viability assessment	>70%
Viable cell recovery		
Identity test	Short tandem repeat	Matches iPSC MCB
Bacterial endotoxin	Kinetic chromogenic LAL (final cell before seeding on substrate)	<5 EU/mL
Mycoplasma	PCR (validated) or PTC method (direct and indirect culture)	No contamination
Sterility test	Gram for fresh product 21 CFR 610.12, bacteriostasis and fungistasis	No contamination
Cell surface markers	Flow cytometry assay for Positive markers: specific for cell type Negative markers: specific for off-target cells	>X% Expression <Y% Expression
Residual undifferentiated iPSCs	Flow cytometry or qPCR assay for expression of pluripotent markers (eg, Oct-3/4, Nanog, Sox2, SSEA-3/4, TRA-1–60/1–81)	<Z%
Potency	Functional assessment of cell performance	Establish by phase 3

iPSC indicates induced pluripotent stem cell; LAL, limulus amoebocyte lysate; MCB, Master Cell Bank; PTC, points to consider; and qPCR, quantitative polymerase chain reaction.

into posterior heart locations may be challenging. Adhesions related to previous sternotomy from past coronary artery bypass surgery, for example, is an additional barrier to this approach. Furthermore, general anesthesia is typically required for these epicardial delivery approaches. Catheter-based transendocardial delivery involves a needle tipped catheter that is advanced from a peripheral artery, retrograde through the aortic valve and into the LV. Although still minimally invasive, this approach offers the ability to target widely and precisely throughout the LV. Imaging systems, such as electroanatomic mapping and multimodality image fusion methods, have been developed to assist catheterization laboratory operators in targeting the site of injections.<sup>105,106</sup> Recent excitement has been generated through applying cellular scaffolds or patches on the cardiac surface via a sternotomy incision or using small incision thoracoscopic techniques.<sup>107,108</sup> It is likely that the optimal cell delivery method will be closely linked to the type of stem cell and its mechanism of therapeutic effect.

Complementing cell delivery strategies are methods to track transplanted cells in vivo. Such tracking enables understanding of cell retention, engraftment, and routes of cell egress from the myocardium after delivery. This information can be used to guide optimization of cell delivery methods, dosing regimens, and timing of dosing including repeat dosing. Various cell labeling and imaging methods have been explored to track transplanted cells and are described in detail elsewhere.<sup>109,110</sup> The sensitivity of detecting the label associated with cells, the spatial resolution of the imaging modality used, and detrimental effects of the labeling procedure on the stem cells vary significantly between cell tracking methods. In general, the iPSC derivatives can be labeled using either direct labeling with image tags (eg, magnetic nanoparticles for MRI) or indirect labels, such as reporter genes (eg, herpes simplex virus thymidine kinase for positron emission tomography). Direct methods of labeling have the advantage of simplicity, and several safe tags have been clinically tested. However, these direct labels can leak out of cells and persist

after cells die, and thus cautious interpretation of the imaging data are required. In contrast, indirect cell labeling typically uses an engineered reporter gene that is incorporated into the cells genome to track living transplanted cells and their cellular progeny, but the challenges associated with this method are related to genetically modifying the cell product. Although optical techniques, including fluorescence and bioluminescence imaging, have proven useful in small animal studies, these studies are of limited value in clinical studies because of light attenuation. Human cardiac cell therapy studies to date have used PET, single photon emission computed topographical, or planar gamma camera imaging for monitoring acute cell retention with directly labeled cells. Studies of long-term cell engraftment and survival have not yet been accomplished clinically, but as techniques to safely genetically engineer stem cell lines improve, such long-term studies are anticipated and will advance the field. iPSCs, like ESCs, may be more amenable to these genetic modifications given their unique ability to proliferate in culture and undergo genome editing.

## Safety Consideration from iPSC-based Therapies

### Tumorigenicity

A hurdle for the use of pluripotent cells in regenerative medicine is their tumorigenic potential, and although this risk is shared for ESCs and iPSCs, there are some distinct risks associated with iPSCs. A shared property of both iPSC and ESC is teratoma formation after injection of undifferentiated cells into immune-compromised mice. Although teratomas are regarded as benign tumors, such tumor formation could be a major problem for clinical applications. Therefore, efforts at removing undifferentiated cells and obtaining pure differentiated cell populations have been pursued. A variety of selection strategies have been investigated, including the use of genetically engineered cell lines to express a cell lineage-specific promoter driving the expression of reporter gene (eg, GFP) or an antibiotic resistance gene.<sup>77,111</sup> In addition, efforts

to identify optimal cell lineages using cell surface markers are being advanced.<sup>71,112</sup> The prolonged culture of ESCs and iPSCs, including scale-up and differentiation processes, provide opportunities for genetic abnormalities to develop which could be tumorigenic. It has also been observed that some ESCs and iPSCs exhibit upregulation in some miRNAs commonly found in cancers.<sup>113</sup>

There are also factors that can specifically affect the risk of tumorigenesis in iPSCs, including the reprogramming process itself and the somatic cell source for reprogramming.<sup>114,115</sup> The initial mouse iPSC lines generated using *c-myc* as one of the reprogramming factors were prone to tumor formation, but even in the absence of *c-myc*, some mouse iPS lines when differentiated into neurospheres formed tumors when transplanted into mouse brains.<sup>68</sup> Another concern is that the starting somatic cells used for reprogramming may have acquired somatic mutations, which will be carried through to the iPSCs. Furthermore, mutations in iPSC lines tend to be concentrated in cancer-related genes.<sup>116</sup> Only long-term studies of transplanted cells in animal models will truly allow assessment of tumorigenesis risk, but the choice of animal models for testing can be complex. Many studies have used immune-compromised animals to allow direct testing of human cells, but it is possible that normal immune surveillance mechanisms will reduce or prevent some tumor risk in the true therapeutic context. Additional research and experience are needed to determine appropriate standards for this testing.

### Genetic Abnormalities

The genetic integrity of hiPSC lines will be critical for safe therapeutic applications. Like human ESCs, hiPSCs can exhibit chromosomal aberrations.<sup>117</sup> In iPSCs, these aberrations can originate with the somatic cell used for reprogramming or as a result of culture adaptation. Routine karyotype analysis is used to identify aneuploid lines and typically exclude them from further development as a potential therapeutic tool. Less commonly, higher resolution evaluation for chromosomal integrity is performed using comparative genomic hybridization or single-nucleotide polymorphism arrays. Most recently, high-throughput sequencing technology has allowed in-depth genetic analysis of iPSC lines and documented the presence of novel point mutations in the iPSC. It is estimated based on a study of 22 hiPSC lines that there are on average 6 protein-coding point mutations per iPSC line.<sup>116</sup> About half of these mutations arose in fibroblasts used for reprogramming at low frequency, whereas the other half occurred during or after the reprogramming process. These spontaneous mutations were found to be clustered in genes commonly mutated in cancer. The functional significance of the point mutations in the cells has not been directly tested as of yet, and how variable different cell lines will be in this regard is unknown. Will each individual iPSC line require exome or whole genome sequencing to confirm safety? Will screening a small panel of key disease causing genes be adequate? A normal karyotype will be required for an iPSC line to be considered for therapeutic applications, but some degree of higher level genetic analysis will also likely become part of standard safety evaluation.

### Immunogenicity of iPSC

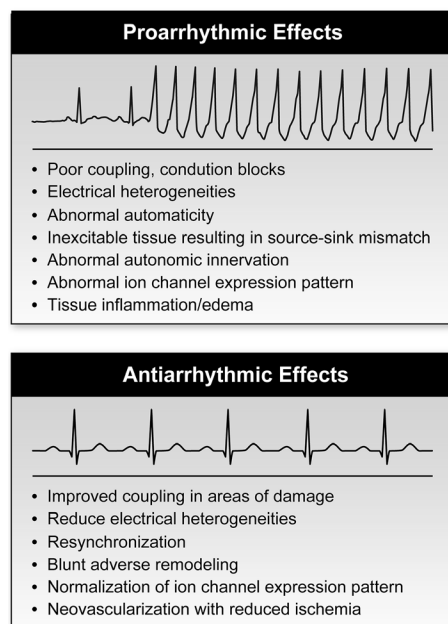
One of the appealing aspects of iPSC-based therapies is that an autologous cell source can be used. It has been generally assumed that autologously derived iPSCs would be immune tolerated by the recipient after transplant. However, Zhao et al<sup>118</sup> first reported immune rejection in autologously transplanted miPSCs. They transplanted ESCs- and MEF-derived iPSCs from B6 mice into syngenic recipients and observed that only iPSC-derived teratomas had T-cell infiltration, a classic sign of immune rejection. iPSC immunogenicity was partly credited to coding sequence mutations (that iPSCs can acquire from their parental somatic cells or during reprogramming) and epigenetic differences between ESCs and iPSCs, which can lead to abnormal antigen expression during iPSCs differentiation. This unexpected finding raised questions about the therapeutic potential of autologous iPSCs. However, this study had some limitations. First, the iPSC lines were compared with only 1 mESC line. To establish that the immunogenicity was specific to iPSCs, more mESCs lines would need to be included in the study because it is well known that mESC lines themselves have diverse differentiation potential. In addition, this study used pluripotent iPSCs for teratomas formation and thus the immune rejection could have been stimulated by tumorigenesis rather than simply the immunogenicity of the iPSCs.<sup>119</sup> Studies testing immunogenicity of iPSC-derived, differentiated cells soon followed. Guha et al<sup>120</sup> and Araki et al<sup>121</sup> reported that miPSC-derived differentiated cells do not illicit an immune response after transplantation in syngenic recipients. Immunogenicity of iPSCs may be reduced by deriving them from less immunogenic somatic cells. Liu et al<sup>122</sup> observed that neural progenitor cells differentiated from umbilical cord-derived iPSCs were less immunogenic when compared with neural progenitor cells differentiated from skin fibroblasts-derived iPSCs.

For transitioning iPSC technology to clinical trials, the immunogenicity of hiPSC-derived cells needs to be examined. The appropriate preclinical model to characterize the immunogenicity of such human cell products is not well established. One approach is to reconstitute severely immunodeficient NOD/SCID-IL2R $\gamma^{-/-}$  (NSG) mice with humanized immune system.<sup>123</sup> In these humanized mice autologous and allogeneic cell products can be characterized for their long-term engraftment or rejection.

### Arrhythmia Risk

One of the serious risks of cardiac cell therapy is potentially lethal ventricular arrhythmias. The post infarction heart is a vulnerable substrate for the generation of ventricular arrhythmias, and theoretically cell therapy could modify this substrate to either lower or raise the risk of clinically significant ventricular arrhythmias (Figure 3).<sup>124,125</sup> The proarrhythmic or antiarrhythmic effects of cell therapies will depend on the cell type(s) transplanted, the integration and coupling of the cells, and the intrinsic properties of the cells. In addition, it is possible that the risk profile will be dynamic potentially changing from proarrhythmic effects to antiarrhythmic effects as repair is affected. Cell therapy can be envisioned as promoting multiple possible arrhythmic mechanisms, such as introducing conduction blocks and electric heterogeneities, thus providing





**Figure 3. Arrhythmia risk from cellular therapies.** Cell therapy has the potential to induce and prevent arrhythmias in the post-myocardial infarction heart. Potential mechanisms for antiarrhythmic and proarrhythmic effects are listed.

substrate for re-entrant arrhythmias. In addition, abnormal automaticity of the transplanted cells could initiate arrhythmias. Triggered activity from abnormal repolarization of the transplanted cardiomyocytes or abnormal calcium cycling could likewise increase the risk of arrhythmias. Alternatively, if the dominant effect of cells is to improve coupling in the areas of damage for resynchronization and repair the heart, the effect could be to reduce arrhythmia risk.

Experimental data in animal models examining specifically the effect of cell therapy after MI on arrhythmia risk are limited. The importance of transplanted cells being able to form connexin 43 containing gap junctions for proper electric integration and low arrhythmic risk has been highlighted by studies transplanting SM, which normally do not express connexin 43 or couple to cardiomyocytes.<sup>126,127</sup> The studies of ESC-derived cardiomyocytes transplanted after MI that have looked specifically at arrhythmia risk have found both proarrhythmic effects in one study and antiarrhythmic effects in a second study of human ESC-derived cardiomyocytes.<sup>128,129</sup> Understanding and resolving these apparently conflicting results will be important for safely advancing these therapies. In addition, the optimal model for assessing arrhythmia risk is not yet clear because rodents lack the cardiac tissue volume present in humans and larger mammals, which may be an essential feature for genesis of some arrhythmias.

Completed clinical trials of cardiac cell therapy in general have not identified major arrhythmia risk at this point. Although an early study of myoblast therapy for ischemic cardiomyopathy found frequent ventricular arrhythmia in treated patients, there was no control group for comparison in this high-risk patient population.<sup>130</sup> None of the phase II randomized trials of bone marrow mononuclear cell therapy after MI have found increased incidence of arrhythmia after cell

therapy, and a meta-analysis of cardiac post-MI cell therapy trials likewise suggested no proarrhythmic effects based on existing data.<sup>131</sup> Nevertheless, the risk of arrhythmias remains a real concern that will merit continued vigilance.

## Remaining Questions for iPSC-Based Clinical Applications

Despite remarkable progress in the understanding of iPSCs since their discovery in 2006 and rapidly advancing technology, there remain major questions in defining the biology of these cells with special relevance to long-term clinical applications. What is the reprogrammed iPSC state and how stable is it? Are iPSCs equivalent to ESCs, and does it matter for clinically relevant cell types? Are differentiated progeny of iPSCs phenotypically and functionally stable or are they prone to further phenotypic changes such as premature senescence? What cell types derived from iPSCs and at what level of maturity are optimal for post-MI therapy? How can long-term survival of the cells be promoted for robust regeneration?

Studies comparing ESCs and iPSCs have pointed to the similarity of the stem cell types, but these studies have also indicated clear differences in gene expression, epigenetics, and miRNA profiles. Functional assays on ESC and iPSCs support comparable pluripotency properties with the formation of teratomas containing derivatives of the 3 primary germ layers. In the case of mouse iPSCs, even more stringent functional tests of pluripotency, such as germline transmission and tetraploid complementation, have been demonstrated for some iPSC lines<sup>132–134</sup>; however, such tests are not possible for hiPSCs. Although iPSCs show much greater similarity in gene expression pattern to ESCs than to the starting cell type that was reprogrammed, multiple studies have demonstrated that iPSCs exhibit clear differences in gene expression when compared with ESCs.<sup>135,136</sup> These differences in gene expression between iPSC and ESC are greatest for low-passage iPSCs, yet some differences persist regardless of passage number.<sup>136</sup> Distinct culture conditions and unique genetic backgrounds can contribute to some of the experimentally observed variations in gene expression between iPSCs and ESCs. In addition, the epigenetic state of iPSC and ESCs, although highly similar, is not identical.<sup>124,137</sup> For example, clear differences in DNA methylation have been described between iPSCs and ESCs. There is evidence of epigenetic memory in iPSCs with retention of some methylation patterns of DNA from the cell of origin.<sup>49,138–141</sup> Thus, the prevailing evidence suggests that although ESCs and iPSCs are both clearly pluripotent stem cells, they are not fully equivalent in all of their characteristics. Whether these differences are problematic or advantageous for therapeutic applications is not known.

How stable and robust are the differentiated cell lineages from iPSCs? For clinical applications, the desired cell type ideally needs to exhibit functional properties appropriate for the long-term reconstitution of the organ. Some studies have raised the question of premature senescence in the case of hematopoietic cells differentiated from iPSCs.<sup>142</sup> In addition, iPSCs exhibit an epigenetic memory that can bias differentiation toward the starting cell lineage.<sup>49</sup> Furthermore, the epigenetic marks retained in iPSCs from the starting cell

lineage can persist in cells differentiated to different lineages.<sup>124,137</sup> Because ESCs have been more extensively characterized and evidence of long-term stable cardiac grafts has been obtained for ESC-derivatives in animal models, comparison between ESC- and iPSC-derived cardiac cell types is an important goal in the field. Our initial characterization of cardiac differentiation of hiPSCs demonstrated that they are highly comparable with ESCs in terms of efficiency in forming contracting EB, expression of cardiac-specific genes during differentiation, proliferation, sarcomeric organization, electrophysiological properties, and chronotropic regulation by the  $\beta$ -adrenergic signaling pathway.<sup>74,143</sup> Recently, Gupta et al<sup>144</sup> and van Laake et al<sup>145</sup> published studies comparing the global transcriptomes of cardiac lineage cells derived from ESCs and iPSCs. They reported that mouse ESC- and iPSC-derived cardiac lineage cells were similarly enriched for cardio-specific genes, and only a minority of the analyzed genes was differentially expressed. The differences in gene expression between ES- and iPS-derived cardiac lineage cells were no greater than differences between cardiac lineage cells derived from different ES cell lines. In spite of minor differences in gene expression, sarcomeric organization, electrophysiological properties, and calcium handling of ES- and iPS-derived cardiomyocytes were indistinguishable.<sup>144</sup> These studies revealed a surprisingly high degree of similarity (transcriptional and functional) between the differentiated cardiac progeny of ES and iPS cells, suggesting that differences between gene expression among pluripotent ES and iPS cells narrow after/during lineage commitment. So if the differentiated progeny of ES and iPS reveal no significant differences, how much consideration should be given for the differences in their pluripotent states? Although these initial *in vitro* comparisons are encouraging, long-term engraftment studies in animal models and particularly large animal models are needed to demonstrate the stability and robustness of the cellular phenotype.

Although a variety of different cellular preparations differentiated from iPSCs have been studied in post-MI animal models as described in Table 2, the optimal cell preparation remains unknown. Few side-by-side comparisons of different cell preparations have been done. Will a pure population of iPS-cardiomyocytes be optimal or is a mixed population including endothelial and smooth muscle cells be better? Alternatively, could delivery of cardiac progenitor cell derived from iPSC provide greatest therapeutic benefit? Defining the optimal cell preparation does not stop at choosing the desired cell type(s), but the functional properties and particularly the maturity of the cells is another important variable because studies have indicated that, at least for iPSC-cardiomyocytes, the phenotype is that of embryonic or fetal cardiomyocytes, not adult. Although there are potential advantages of transplanting more fetal-like cardiomyocytes, including their smaller size, relative resistance to ischemia and oxidative stress, there are potential disadvantages such as their automaticity that could trigger arrhythmias and lack of mature excitation-contraction coupling, which could limit their functional contractile output. Again additional studies are needed particularly in large animal models, which can provide comparisons of different cell

preparations. Ideally, a standardized large animal model could facilitate comparisons between different studies as well.

If the greatest promise of iPSC products is to truly regenerate the myocardium with transplanted cells functionally integrating into the heart, then the long-term survival of the donor cells is essential. However, most studies that have examined long-term survival of ESC- and iPSC-derived cell preparations have shown that the number of surviving cells diminishes logarithmically over days or weeks.<sup>17,93,146</sup> The reasons for this cell loss over time are not clear but may relate to the hostile environment in the post-MI remodeling heart, lack of adequate perfusion, slow immune rejection, or intrinsic properties of the transplanted cells limiting their ability to adapt and survive in the native heart. A variety of approaches have been tested to improve survival, such as injecting ESC-cardiomyocytes with a prosurvival cocktail containing a variety of growth factors, antiapoptotic agents, and antioxidants.<sup>17</sup> An alternative approach is to use tissue engineering principles to generate scaffolds for optimal cell delivery and survival, which ideally will integrate into the myocardium.<sup>108</sup> Perhaps transplanting the right combination of cells could promote long-term survival as suggested by a study in a porcine MI model in which cotransplanting hMSCs promoted the survival of hiPSCs derivatives for  $\geq 15$  weeks.<sup>93</sup> These early studies provide hope that robust myocardial regeneration may be possible in the future with continued optimization and innovation.

## Conclusions

Overall, iPSC technology has opened up new approaches for cell-based therapies after MI. There are appealing aspects of this cell source, including autologous cell products, large banks of well-characterized genetically defined cells, and a wide range of possible therapeutic cellular products. Preclinical studies primarily in small animals and beginning studies in larger animals have shown promising initial results. Clinical experience with iPSCs is beginning with the first-in-man trial of an iPSC product transplanting autologous retinal pigment epithelial cells to treat age-related macular degeneration in Japan. Cardiovascular clinical applications will likely follow as ongoing research defines the optimal cell preparations and best delivery strategies, as well as ensures safety.

## Acknowledgments

We acknowledge the outstanding contributions of many researchers in the field whose work could not be directly cited because of space constraints.

## Sources of Funding

Dr Kamp receives funding from the National Institutes of Health from grants U01HL099773, R01EB007534, and R01 HL108670. P.A. Lalit is recipient of an American Heart Association Predoctoral Fellowship Award 12PRE9520035. D.J. Hei and A.N. Raval receive funding from National Heart, Lung, and Blood Institute contract no. HHSN268201000010C (PACT).

## Disclosures

Dr Kamp is a founding shareholder and consultant for Cellular Dynamics International which produces induced pluripotent stem cells products for drug discovery, toxicity testing, and research applications.

## References

- Leobon B, Garcin I, Menasche P, Vilquin JT, Audinat E, Charpak S. Myoblasts transplanted into rat infarcted myocardium are functionally isolated from their host. *Proc Natl Acad Sci U S A*. 2003;100:7808–7811.
- Orlic D, Kajstura J, Chimenti S, Jakoniuk I, Anderson SM, Li B, Pickel J, McKay R, Nadal-Ginard B, Bodine DM, Leri A, Anversa P. Bone marrow cells regenerate infarcted myocardium. *Nature*. 2001;410:701–705.
- Murry CE, Soonpaa MH, Reinecke H, Nakajima H, Nakajima HO, Rubart M, Pasumarthi KB, Virag JJ, Bartelmez SH, Poppa V, Bradford G, Dowell JD, Williams DA, Field LJ. Haematopoietic stem cells do not transdifferentiate into cardiac myocytes in myocardial infarcts. *Nature*. 2004;428:664–668.
- Balsam LB, Wagers AJ, Christensen JL, Kofidis T, Weissman IL, Robbins RC. Haematopoietic stem cells adopt mature haematopoietic fates in ischaemic myocardium. *Nature*. 2004;428:668–673.
- Lunde K, Solheim S, Aakhus S, et al. Intracoronary injection of mononuclear bone marrow cells in acute myocardial infarction. *N Engl J Med*. 2006;355:1199–1209.
- Schächinger V, Erbs S, Elsässer A, Haberbosch W, Hambrecht R, Holschermann H, Yu J, Corti R, Mathey DG, Hamm CW, Süselbeck T, Assmus B, Tonn T, Dimmeler S, Zeiher AM; REPAIR-AMI Investigators. Intracoronary bone marrow-derived progenitor cells in acute myocardial infarction. *N Engl J Med*. 2006;355:1210–1221.
- Beltrami AP, Barlucchi L, Torella D, Baker M, Limana F, Chimenti S, Kasahara H, Rota M, Musso E, Urbanek K, Leri A, Kajstura J, Nadal-Ginard B, Anversa P. Adult cardiac stem cells are multipotent and support myocardial regeneration. *Cell*. 2003;114:763–776.
- Oh H, Bradfute SB, Gallardo TD, Nakamura T, Gaussen V, Mishina Y, Pocius J, Michael LH, Behringer RR, Garry DJ, Entman ML, Schneider MD. Cardiac progenitor cells from adult myocardium: homing, differentiation, and fusion after infarction. *Proc Natl Acad Sci U S A*. 2003;100:12313–12318.
- Bolli R, Chugh AR, D'Amario D, et al. Cardiac stem cells in patients with ischaemic cardiomyopathy (SCIPIO): initial results of a randomised phase 1 trial. *Lancet*. 2011;378:1847–1857.
- Makkar RR, Smith RR, Cheng K, Malliaras K, Thomson LE, Berman D, Czer LS, Marbán L, Mendizabal A, Johnston PV, Russell SD, Schuleri KH, Lardo AC, Gerstenblith G, Marbán E. Intracoronary cardiosphere-derived cells for heart regeneration after myocardial infarction (CADUCEUS): a prospective, randomised phase 1 trial. *Lancet*. 2012;379:895–904.
- Zaruba MM, Soonpaa M, Kong D, He H, Rezvani M, Ingwall JS, Dzau VJ. Mesenchymal stem cells modified with Akt prevent remodeling and restore performance of infarcted hearts. *Nat Med*. 2003;9:1195–1201.
- Mirotsov M, Zhang Z, Deb A, Zhang L, Gnechhi M, Noiseux N, Mu H, Pachori A, Dzau V. Secreted frizzled related protein 2 (Sfrp2) is the key Akt-mesenchymal stem cell-released paracrine factor mediating myocardial survival and repair. *Proc Natl Acad Sci U S A*. 2007;104:1643–1648.
- Hare JM, Traverse JH, Henry TD, Dib N, Strumpf RK, Schulman SP, Gerstenblith G, DeMaria AN, Denktas AE, Gammon RS, Hermiller JB Jr, Reisman MA, Schaer GL, Sherman W. A randomized, double-blind, placebo-controlled, dose-escalation study of intravenous adult human mesenchymal stem cells (prochymal) after acute myocardial infarction. *J Am Coll Cardiol*. 2009;54:2277–2286.
- Bartunek J, Behfar A, Dolatabadi D, et al. Cardiopoietic stem cell therapy in heart failure: the C-CURE (Cardiopoietic stem Cell therapy in heart failure) multicenter randomized trial with lineage-specified biologics. *J Am Coll Cardiol*. 2013;61:2329–2338.
- Laflamme MA, Chen KY, Naumova AV, et al. Cardiomyocytes derived from human embryonic stem cells in pro-survival factors enhance function of infarcted rat hearts. *Nat Biotechnol*. 2007;25:1015–1024.
- Dai W, Field LJ, Rubart M, Reuter S, Hale SL, Zweigerdt R, Graichen RE, Kay GL, Jyrala AJ, Colman A, Davidson BP, Pera M, Klöner RA. Survival and maturation of human embryonic stem cell-derived cardiomyocytes in rat hearts. *J Mol Cell Cardiol*. 2007;43:504–516.
- Kehat I, Khimovich L, Caspi O, Gepstein L, Shofti R, Arbel G, Huber I, Satin J, Itskovitz-Eldor J, Gepstein L. Electromechanical integration of cardiomyocytes derived from human embryonic stem cells. *Nat Biotechnol*. 2004;22:1282–1289.
- Singla DK, Hacker TA, Ma L, Douglas PS, Sullivan R, Lyons GE, Kamp TJ. Transplantation of embryonic stem cells into the infarcted mouse heart: formation of multiple cell types. *J Mol Cell Cardiol*. 2006;40:195–200.
- Singla DK, Lyons GE, Kamp TJ. Transplanted embryonic stem cells following mouse myocardial infarction inhibit apoptosis and cardiac remodeling. *Am J Physiol Heart Circ Physiol*. 2007;293:H1308–H1314.
- Min JY, Yang Y, Sullivan MF, Ke Q, Converso KL, Chen Y, Morgan JP, Xiao YF. Long-term improvement of cardiac function in rats after infarction by transplantation of embryonic stem cells. *J Thorac Cardiovasc Surg*. 2003;125:361–369.
- Min JY, Yang Y, Converso KL, Liu L, Huang Q, Morgan JP, Xiao YF. Transplantation of embryonic stem cells improves cardiac function in postinfarcted rats. *J Appl Physiol (1985)*. 2002;92:288–296.
- Ménard C, Hagege AA, Agbulut O, Barro M, Morichetti MC, Brasselet C, Bel A, Messas E, Bissery A, Bruneval P, Desnos M, Pucéat M, Menasché P. Transplantation of cardiac-committed mouse embryonic stem cells to infarcted sheep myocardium: a preclinical study. *Lancet*. 2005;366:1005–1012.
- Hodgson DM, Behfar A, Zingman LV, Kane GC, Perez-Terzic C, Alekseev AE, Pucéat M, Terzic A. Stable benefit of embryonic stem cell therapy in myocardial infarction. *Am J Physiol Heart Circ Physiol*. 2004;287:H471–H479.
- Nussbaum J, Minami E, Laflamme MA, Virag JA, Ware CB, Masino A, Muskheili V, Pabon L, Reinecke H, Murry CE. Transplantation of undifferentiated murine embryonic stem cells in the heart: teratoma formation and immune response. *FASEB J*. 2007;21:1345–1357.
- Guan K, Wagner S, Unsöld B, Maier LS, Kaiser D, Hemmerlein B, Nayernia K, Engel W, Hasenfuss G. Generation of functional cardiomyocytes from adult mouse spermatogonial stem cells. *Circ Res*. 2007;100:1615–1625.
- Didié M, Christalla P, Rubart M, et al. Parthenogenetic stem cells for tissue-engineered heart repair. *J Clin Invest*. 2013;123:1285–1298.
- Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell*. 2006;126:663–676.
- Ieda M, Fu JD, Delgado-Olguin P, Vedantham V, Hayashi Y, Bruneau BG, Srivastava D. Direct reprogramming of fibroblasts into functional cardiomyocytes by defined factors. *Cell*. 2010;142:375–386.
- Takahashi K, Tanabe K, Ohnuki M, Narita M, Ichisaka T, Tomoda K, Yamanaka S. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell*. 2007;131:861–872.
- Yu J, Vodyanik MA, Smuga-Otto K, Antosiewicz-Bourget J, Frane JL, Tian S, Nie J, Jonsdottir GA, Ruotti V, Stewart R, Slukvin II, Thomson JA. Induced pluripotent stem cell lines derived from human somatic cells. *Science*. 2007;318:1917–1920.
- Qian L, Huang Y, Spencer CI, Foley A, Vedantham V, Liu L, Conway SJ, Fu JD, Srivastava D. In vivo reprogramming of murine cardiac fibroblasts into induced cardiomyocytes. *Nature*. 2012;485:593–598.
- Inagawa K, Miyamoto K, Yamakawa H, Muraoka N, Sadahiro T, Umei T, Wada R, Katsumata Y, Kaneda R, Nakade K, Kurihara C, Obata Y, Miyake K, Fukuda K, Ieda M. Induction of cardiomyocyte-like cells in infarct hearts by gene transfer of Gata4, Mef2c, and Tbx5. *Circ Res*. 2012;111:1147–1156.
- Song K, Nam YJ, Luo X, Qi X, Tan W, Huang GN, Acharya A, Smith CL, Tallquist MD, Neilson EG, Hill JA, Bassel-Duby R, Olson EN. Heart repair by reprogramming non-myocytes with cardiac transcription factors. *Nature*. 2012;485:599–604.
- Yu J, Hu K, Smuga-Otto K, Tian S, Stewart R, Slukvin II, Thomson JA. Human induced pluripotent stem cells free of vector and transgene sequences. *Science*. 2009;324:797–801.
- Hochedlinger K, Plath K. Epigenetic reprogramming and induced pluripotency. *Development*. 2009;136:509–523.
- Jaenisch R, Young R. Stem cells, the molecular circuitry of pluripotency and nuclear reprogramming. *Cell*. 2008;132:567–582.
- Seki T, Yuasa S, Oda M, Egashira T, Yae K, Kusumoto D, Nakata H, Tohyama S, Hashimoto H, Kodaira M, Okada Y, Seimiya H, Fusaki N, Hasegawa M, Fukuda K. Generation of induced pluripotent stem cells from human terminally differentiated circulating T cells. *Cell Stem Cell*. 2010;7:11–14.
- Brown ME, Rondon E, Rajesh D, Mack A, Lewis R, Feng X, Ziturs LJ, Learch RD, Nuwaysir EF. Derivation of induced pluripotent stem cells from human peripheral blood T lymphocytes. *PLoS One*. 2010;5:e11373.
- Bunnell BA, Flaat M, Gagliardi C, Patel B, Ripoll C. Adipose-derived stem cells: isolation, expansion and differentiation. *Methods*. 2008;45:115–120.



42. Sun N, Panetta NJ, Gupta DM, Wilson KD, Lee A, Jia F, Hu S, Cherry AM, Robbins RC, Longaker MT, Wu JC. Feeder-free derivation of induced pluripotent stem cells from adult human adipose stem cells. *Proc Natl Acad Sci U S A*. 2009;106:15720–15725.
43. Giorgetti A, Montserrat N, Aasen T, Gonzalez F, Rodríguez-Piñá I, Vassena R, Raya A, Boué S, Barrero MJ, Corbella BA, Torradabellà M, Veiga A, Izpisua Belmonte JC. Generation of induced pluripotent stem cells from human cord blood using OCT4 and SOX2. *Cell Stem Cell*. 2009;5:353–357.
44. Liu T, Zou G, Gao Y, Zhao X, Wang H, Huang Q, Jiang L, Guo L, Cheng W. High efficiency of reprogramming cd34+ cells derived from human amniotic fluid into induced pluripotent stem cells with oct4. *Stem Cells Dev*. 2012;21(12):2322–2332.
45. Zhou T, Benda C, Duzinger S, et al. Generation of induced pluripotent stem cells from urine. *J Am Soc Nephrol*. 2011;22:1221–1228.
46. Aasen T, Raya A, Barrero MJ, Garreta E, Consiglio A, Gonzalez F, Vassena R, Bilić J, Pekarik V, Tiscornia G, Edel M, Boué S, Izpisua Belmonte JC. Efficient and rapid generation of induced pluripotent stem cells from human keratinocytes. *Nat Biotechnol*. 2008;26:1276–1284.
47. Eminli S, Foudi A, Stadtfeld M, Maherali N, Ahfeldt T, Mostoslavsky G, Hock H, Hochedlinger K. Differentiation stage determines potential of hematopoietic cells for reprogramming into induced pluripotent stem cells. *Nat Genet*. 2009;41:968–976.
48. Pasha Z, Haider HKH, Ashraf M. Efficient non-viral reprogramming of myoblasts to stemness with a single small molecule to generate cardiac progenitor cells. *PLoS One*. 2011;6:e23667.
49. Kim K, Doi A, Wen B, et al. Epigenetic memory in induced pluripotent stem cells. *Nature*. 2010;467:285–290.
50. Ban H, Nishishita N, Fusaki N, Tabata T, Saeki K, Shikamura M, Takada N, Inoue M, Hasegawa M, Kawamata S, Nishikawa S. Efficient generation of transgene-free human induced pluripotent stem cells (iPSCs) by temperature-sensitive Sendai virus vectors. *Proc Natl Acad Sci U S A*. 2011;108:14234–14239.
51. Okita K, Hong H, Takahashi K, Yamanaka S. Generation of mouse-induced pluripotent stem cells with plasmid vectors. *Nat Protoc*. 2010;5:418–428.
52. Jia F, Wilson KD, Sun N, Gupta DM, Huang M, Li Z, Panetta NJ, Chen ZY, Robbins RC, Kay MA, Longaker MT, Wu JC. A nonviral minicircle vector for deriving human iPS cells. *Nat Methods*. 2010;7:197–199.
53. Yusa K, Rad R, Takeda J, Bradley A. Generation of transgene-free induced pluripotent mouse stem cells by the piggyBac transposon. *Nat Methods*. 2009;6:363–369.
54. Wolffen K, Michael IP, Mohseni P, Desai R, Mileikovsky M, Hämmäläinen R, Cowling R, Wang W, Liu P, Gertsenstein M, Kaji K, Sung HK, Nagy A. piggyBac transposition reprograms fibroblasts to induced pluripotent stem cells. *Nature*. 2009;458:766–770.
55. Kim D, Kim CH, Moon JI, Chung YG, Chang MY, Han BS, Ko S, Yang E, Cha KY, Lanza R, Kim KS. Generation of human induced pluripotent stem cells by direct delivery of reprogramming proteins. *Cell Stem Cell*. 2009;4:472–476.
56. Warren L, Manos PD, Ahfeldt T, et al. Highly efficient reprogramming to pluripotency and directed differentiation of human cells with synthetic modified mRNA. *Cell Stem Cell*. 2010;7:618–630.
57. Lee J, Sayed N, Hunter A, Au KF, Wong WH, Mocarski ES, Pera RR, Yakubov E, Cooke JP. Activation of innate immunity is required for efficient nuclear reprogramming. *Cell*. 2012;151:547–558.
58. Huangfu D, Maehr R, Guo W, Eijkelenboom A, Snitow M, Chen AE, Melton DA. Induction of pluripotent stem cells by defined factors is greatly improved by small-molecule compounds. *Nat Biotechnol*. 2008;26:795–797.
59. Mercola M, Colas A, Willems E. Induced pluripotent stem cells in cardiovascular drug discovery. *Circ Res*. 2013;112:534–548.
60. Anson BD, Kolaja KL, Kamp TJ. Opportunities for use of human iPS cells in predictive toxicology. *Clin Pharmacol Ther*. 2011;89:754–758.
61. Olson EN. Gene regulatory networks in the evolution and development of the heart. *Science*. 2006;313:1922–1927.
62. Evans SM, Yelon D, Conlon FL, Kirby ML. Myocardial lineage development. *Circ Res*. 2010;107:1428–1444.
63. Mummery C, Ward-van Oostwaard D, Doevendans P, Spijker R, van den Brink S, Hassink R, van der Heyden M, Opthof T, Pera M, de la Riviere AB, Passier R, Tertoolen L. Differentiation of human embryonic stem cells to cardiomyocytes: role of coculture with visceral endoderm-like cells. *Circulation*. 2003;107:2733–2740.
64. Mummery CL, Zhang J, Ng ES, Elliott DA, Elefanti AG, Kamp TJ. Differentiation of human embryonic stem cells and induced pluripotent stem cells to cardiomyocytes: a methods overview. *Circ Res*. 2012;111:344–358.
65. Lian X, Hsiao C, Wilson G, Zhu K, Hazeltine LB, Azarin SM, Raval KK, Zhang J, Kamp TJ, Palecek SP. Robust cardiomyocyte differentiation from human pluripotent stem cells via temporal modulation of canonical Wnt signaling. *Proc Natl Acad Sci U S A*. 2012;109:E1848–E1857.
66. Veraitch O, Kobayashi T, Imaizumi Y, Akamatsu W, Sasaki T, Yamanaka S, Amagai M, Okano H, Ohyama M. Human induced pluripotent stem cell-derived ectodermal precursor cells contribute to hair follicle morphogenesis in vivo. *J Invest Dermatol*. 2013;133:1479–1488.
67. Wanjaré M, Kuo F, Gerecht S. Derivation and maturation of synthetic and contractile vascular smooth muscle cells from human pluripotent stem cells. *Cardiovasc Res*. 2013;97:321–330.
68. Okano H, Nakamura M, Yoshida K, Okada Y, Tsuji O, Nori S, Ikeda E, Yamanaka S, Miura K. Steps toward safe cell therapy using induced pluripotent stem cells. *Circ Res*. 2013;112:523–533.
69. Hill KL, Obrtlíkova P, Alvarez DF, King JA, Keirstead SA, Allred JR, Kaufman DS. Human embryonic stem cell-derived vascular progenitor cells capable of endothelial and smooth muscle cell function. *Exp Hematol*. 2010;38:246–257.e241.
70. Kattman SJ, Witty AD, Gagliardi M, Dubois NC, Niapour M, Hotta A, Ellis J, Keller G. Stage-specific optimization of activin/nodal and BMP signaling promotes cardiac differentiation of mouse and human pluripotent stem cell lines. *Cell Stem Cell*. 2011;8:228–240.
71. Dubois NC, Craft AM, Sharma P, Elliott DA, Stanley EG, Elefanti AG, Gramolini A, Keller G. SIRPA is a specific cell-surface marker for isolating cardiomyocytes derived from human pluripotent stem cells. *Nat Biotechnol*. 2011;29:1011–1018.
72. Trivedi P, Hematti P. Derivation and immunological characterization of mesenchymal stromal cells from human embryonic stem cells. *Exp Hematol*. 2008;36:350–359.
73. Hare JM. Translational development of mesenchymal stem cell therapy for cardiovascular diseases. *Tex Heart Inst J*. 2009;36:145–147.
74. Zhang J, Wilson GF, Soerens AG, Koonce CH, Yu J, Palecek SP, Thomson JA, Kamp TJ. Functional cardiomyocytes derived from human induced pluripotent stem cells. *Circ Res*. 2009;104:e30–e41.
75. Rufaihah AJ, Huang NF, Kim J, Herold J, Volz KS, Park TS, Lee JC, Zambidis ET, Reijo-Pera R, Cooke JP. Human induced pluripotent stem cell-derived endothelial cells exhibit functional heterogeneity. *Am J Transl Res*. 2013;5:21–35.
76. Elliott DA, Braam SR, Koutsis K, et al. NKX2-5(eGFP/w) hESCs for isolation of human cardiac progenitors and cardiomyocytes. *Nat Methods*. 2011;8:1037–1040.
77. Klug MG, Soonpaa MH, Koh GY, Field LJ. Genetically selected cardiomyocytes from differentiating embryonic stem cells form stable intracardiac grafts. *J Clin Invest*. 1996;98:216–224.
78. Huber I, Itzhaki I, Caspi O, Arbel G, Izukerman M, Gepstein A, Habib M, Yankelson L, Kehat I, Gepstein L. Identification and selection of cardiomyocytes during human embryonic stem cell differentiation. *FASEB J*. 2007;21:2551–2563.
79. Tohyama S, Hattori F, Sano M, et al. Distinct metabolic flow enables large-scale purification of mouse and human pluripotent stem cell-derived cardiomyocytes. *Cell Stem Cell*. 2013;12:127–137.
80. Nelson TJ, Martinez-Fernandez A, Yamada S, Perez-Terzic C, Ikeda Y, Terzic A. Repair of acute myocardial infarction by human stemness factors induced pluripotent stem cells. *Circulation*. 2009;120:408–416.
81. Singla DK, Long X, Glass C, Singla RD, Yan B. Induced pluripotent stem (iPS) cells repair and regenerate infarcted myocardium. *Mol Pharm*. 2011;8:1573–1581.
82. Ahmed RP, Ashraf M, Buccini S, Shujia J, Haider HKH. Cardiac tumorigenic potential of induced pluripotent stem cells in an immunocompetent host with myocardial infarction. *Regen Med*. 2011;6:171–178.
83. Zhang Y, Wang D, Chen M, Yang B, Zhang F, Cao K. Intramyocardial transplantation of undifferentiated rat induced pluripotent stem cells causes tumorigenesis in the heart. *PLoS One*. 2011;6:e19012.
84. Ahmed RP, Haider HK, Buccini S, Li L, Jiang S, Ashraf M. Reprogramming of skeletal myoblasts for induction of pluripotency for tumor-free cardiomyogenesis in the infarcted heart. *Circ Res*. 2011;109:60–70.
85. Buccini S, Haider KH, Ahmed RP, Jiang S, Ashraf M. Cardiac progenitors derived from reprogrammed mesenchymal stem cells contribute to angiogenic repair of the infarcted heart. *Basic Res Cardiol*. 2012;107:301.
86. Dai B, Huang W, Xu M, Millard RW, Gao MH, Hammond HK, Menick DR, Ashraf M, Wang Y. Reduced collagen deposition in infarcted myocardium facilitates induced pluripotent stem cell engraftment and angiomyogenesis for improvement of left ventricular function. *J Am Coll Cardiol*. 2011;58:2118–2127.



87. Tokunaga M, Liu ML, Nagai T, Iwanaga K, Matsuura K, Takahashi T, Kanda M, Kondo N, Wang P, Naito AT, Komuro I. Implantation of cardiac progenitor cells using self-assembling peptide improves cardiac function after myocardial infarction. *J Mol Cell Cardiol*. 2010;49:972–983.
88. Moretti A, Caron L, Nakano A, Lam JT, Bernshausen A, Chen Y, Qyang Y, Bu L, Sasaki M, Martin-Puig S, Sun Y, Evans SM, Laugwitz KL, Chien KR. Multipotent embryonic isl1+ progenitor cells lead to cardiac, smooth muscle, and endothelial cell diversification. *Cell*. 2006;127:1151–1165.
89. Lam JT, Moretti A, Laugwitz KL. Multipotent progenitor cells in regenerative cardiovascular medicine. *Pediatr Cardiol*. 2009;30:690–698.
90. Mauritz C, Martens A, Rojas SV, Schnick T, Rathert C, Schecker N, Menke S, Glage S, Zweigerdt R, Haverich A, Martin U, Kutschka I. Induced pluripotent stem cell (iPSC)-derived Flk-1 progenitor cells engraft, differentiate, and improve heart function in a mouse model of acute myocardial infarction. *Eur Heart J*. 2011;32:2634–2641.
91. Kattman SJ, Huber TL, Keller GM. Multipotent flk-1+ cardiovascular progenitor cells give rise to the cardiomyocyte, endothelial, and vascular smooth muscle lineages. *Dev Cell*. 2006;11:723–732.
92. Kouskoff V, Lacada G, Schwant S, Fehling HJ, Keller G. Sequential development of hematopoietic and cardiac mesoderm during embryonic stem cell differentiation. *Proc Natl Acad Sci U S A*. 2005;102:13170–13175.
93. Templin C, Zweigerdt R, Schwanke K, et al. Transplantation and tracking of human-induced pluripotent stem cells in a pig model of myocardial infarction: Assessment of cell survival, engraftment, and distribution by hybrid single photon emission computed tomography/computed tomography of sodium iodide symporter transgene expression. *Circulation*. 2012;126:430–439.
94. Gneocchi M, He H, Melo LG, Noiseux N, Morello F, de Boer RA, Zhang L, Pratt RE, Dzau VJ, Ingwall JS. Early beneficial effects of bone marrow-derived mesenchymal stem cells overexpressing Akt on cardiac metabolism after myocardial infarction. *Stem Cells*. 2009;27:971–979.
95. Zhao S, Wehner R, Bornhäuser M, Wassmuth R, Bachmann M, Schmitz M. Immunomodulatory properties of mesenchymal stromal cells and their therapeutic consequences for immune-mediated disorders. *Stem Cells Dev*. 2010;19:607–614.
96. Kawamura M, Miyagawa S, Miki K, Saito A, Fukushima S, Higuchi T, Kawamura T, Kuratani T, Daimon T, Shimizu T, Okano T, Sawa Y. Feasibility, safety, and therapeutic efficacy of human induced pluripotent stem cell-derived cardiomyocyte sheets in a porcine ischemic cardiomyopathy model. *Circulation*. 2012;126:S29–S37.
97. Xiong Q, Ye L, Zhang P, Lepley M, Tian J, Li J, Zhang L, Swingen C, Vaughan JT, Kaufman DS, Zhang J. Functional consequences of human induced pluripotent stem cell therapy: myocardial ATP turnover rate in the in vivo swine heart with postinfarction remodeling. *Circulation*. 2013;127:997–1008.
98. Gu M, Nguyen PK, Lee AS, et al. Microfluidic single-cell analysis shows that porcine induced pluripotent stem cell-derived endothelial cells improve myocardial function by paracrine activation. *Circ Res*. 2012;111:882–893.
99. Okita K, Matsumura Y, Sato Y, et al. A more efficient method to generate integration-free human iPS cells. *Nat Methods*. 2011;8:409–412.
100. Chen G, Gulbranson DR, Hou Z, et al. Chemically defined conditions for human iPSC derivation and culture. *Nat Methods*. 2011;8:424–429.
101. Nagaoka M, Si-Tayeb K, Akaike T, Duncan SA. Culture of human pluripotent stem cells using completely defined conditions on a recombinant E-cadherin substratum. *BMC Dev Biol*. 2010;10:60.
102. Raval AN. Therapeutic potential of adult progenitor cells in the management of chronic myocardial ischemia. *Am J Cardiovasc Drugs*. 2008;8:315–326.
103. Campbell NG, Suzuki K. Cell delivery routes for stem cell therapy to the heart: current and future approaches. *J Cardiovasc Transl Res*. 2012;5:713–726.
104. Barbash IM, Chouraqui P, Baron J, Feinberg MS, Etzion S, Tessone A, Miller L, Guetta E, Zipori D, Kedes LH, Kloner RA, Leor J. Systemic delivery of bone marrow-derived mesenchymal stem cells to the infarcted myocardium: feasibility, cell migration, and body distribution. *Circulation*. 2003;108:863–868.
105. Gyöngyösi M, Dib N. Diagnostic and prognostic value of 3D NOGA mapping in ischemic heart disease. *Nat Rev Cardiol*. 2011;8:393–404.
106. Tomkowiak MT, Klein AJ, Vigen KK, Hacker TA, Speidel MA, VanLysel MS, Raval AN. Targeted transcatheter therapeutic delivery guided by MRI-x-ray image fusion. *Catheter Cardiovasc Interv*. 2011;78:468–478.
107. Kreutziger KL, Murry CE. Engineered human cardiac tissue. *Pediatr Cardiol*. 2011;32:334–341.
108. Stevens KR, Kreutziger KL, Dupras SK, Korte FS, Regnier M, Muskheili V, Nourse MB, Bendixen K, Reinecke H, Murry CE. Physiological function and transplantation of scaffold-free and vascularized human cardiac muscle tissue. *Proc Natl Acad Sci U S A*. 2009;106:16568–16573.
109. Nguyen PK, Lan F, Wang Y, Wu JC. Imaging: guiding the clinical translation of cardiac stem cell therapy. *Circ Res*. 2011;109:962–979.
110. Cai W, Zhang Y, Kamp TJ. Imaging of Induced Pluripotent Stem Cells: From Cellular Reprogramming to Transplantation. *Am J Nucl Med Mol Imaging*. 2011;1:18–28.
111. Metzger JM, Lin WI, Samuelson LC. Vital staining of cardiac myocytes during embryonic stem cell cardiogenesis in vitro. *Circ Res*. 1996;78:547–552.
112. Uosaki H, Fukushima H, Takeuchi A, Matsuoka S, Nakatsuji N, Yamanaka S, Yamashita JK. Efficient and scalable purification of cardiomyocytes from human embryonic and induced pluripotent stem cells by VCAM1 surface expression. *PLoS One*. 2011;6:e23657.
113. Neveu P, Kye MJ, Qi S, Buchholz DE, Clegg DO, Sahin M, Park IH, Kim KS, Daley GQ, Kornblum HI, Shraiman BI, Kosik KS. MicroRNA profiling reveals two distinct p53-related human pluripotent stem cell states. *Cell Stem Cell*. 2010;7:671–681.
114. Ben-David U, Benvenisty N. The tumorigenicity of human embryonic and induced pluripotent stem cells. *Nat Rev Cancer*. 2011;11:268–277.
115. Lee AS, Tang C, Rao MS, Weissman IL, Wu JC. Tumorigenicity as a clinical hurdle for pluripotent stem cell therapies. *Nat Med*. 2013;19:998–1004.
116. Gore A, Li Z, Fung HL, et al. Somatic coding mutations in human induced pluripotent stem cells. *Nature*. 2011;471:63–67.
117. Maysar Y, Ben-David U, Lavon N, Biancotti JC, Yakir B, Clark AT, Plath K, Lowry WE, Benvenisty N. Identification and classification of chromosomal aberrations in human induced pluripotent stem cells. *Cell Stem Cell*. 2010;7:521–531.
118. Zhao T, Zhang ZN, Rong Z, Xu Y. Immunogenicity of induced pluripotent stem cells. *Nature*. 2011;474:212–215.
119. Okita K, Nagata N, Yamanaka S. Immunogenicity of induced pluripotent stem cells. *Circ Res*. 2011;109:720–721.
120. Guha P, Morgan JW, Mostoslavsky G, Rodrigues NP, Boyd AS. Lack of immune response to differentiated cells derived from syngeneic induced pluripotent stem cells. *Cell Stem Cell*. 2013;12:407–412.
121. Araki R, Uda M, Hoki Y, Sunayama M, Nakamura M, Ando S, Sugiyama M, Ideno H, Shimada A, Nifuji A, Abe M. Negligible immunogenicity of terminally differentiated cells derived from induced pluripotent or embryonic stem cells. *Nature*. 2013;494:100–104.
122. Liu P, Chen S, Li X, Qin L, Huang K, Wang L, Huang W, Li S, Jia B, Zhong M, Pan G, Cai J, Pei D. Low immunogenicity of neural progenitor cells differentiated from induced pluripotent stem cells derived from less immunogenic somatic cells. *PLoS One*. 2013;8:e69617.
123. Shultz LD, Lyons BL, Burzenski LM, Gott B, Chen X, Chaleff S, Kotb M, Gillies SD, King M, Mangada J, Greiner DL, Handgretinger R. Human lymphoid and myeloid cell development in NOD/LtSz-scid IL2R gamma null mice engrafted with mobilized human hemopoietic stem cells. *J Immunol*. 2005;174:6477–6489.
124. Ruiz S, Diep D, Gore A, et al. Identification of a specific reprogramming-associated epigenetic signature in human induced pluripotent stem cells. *Proc Natl Acad Sci U S A*. 2012;109:16196–16201.
125. Ly HQ, Nattel S. Stem cells are not proarrhythmic: letting the genie out of the bottle. *Circulation*. 2009;119:1824–1831.
126. Roell W, Lewalter T, Sasse P, et al. Engraftment of connexin 43-expressing cells prevents post-infarct arrhythmia. *Nature*. 2007;450:819–824.
127. Chang MG, Tung L, Sekar RB, Chang CY, Cysyk J, Dong P, Marbán E, Abraham MR. Proarrhythmic potential of mesenchymal stem cell transplantation revealed in an in vitro coculture model. *Circulation*. 2006;113:1832–1841.
128. Liao SY, Liu Y, Siu CW, Zhang Y, Lai WH, Au KW, Lee YK, Chan YC, Yip PM, Wu EX, Wu Y, Lau CP, Li RA, Tse HF. Proarrhythmic risk of embryonic stem cell-derived cardiomyocyte transplantation in infarcted myocardium. *Heart Rhythm*. 2010;7:1852–1859.
129. Shiba Y, Fernandes S, Zhu WZ, et al. Human ES-cell-derived cardiomyocytes electrically couple and suppress arrhythmias in injured hearts. *Nature*. 2012;489:322–325.
130. Menasché P, Hagege AA, Vilquin JT, Desnos M, Abergel E, Pouzet B, Bel A, Sarateanu S, Scorsin M, Schwartz K, Bruneval P, Benbunan M, Marolleau JP, Duboc D. Autologous skeletal myoblast transplantation

- for severe postinfarction left ventricular dysfunction. *J Am Coll Cardiol*. 2003;41:1078–1083.
131. Clifford DM, Fisher SA, Brunskill SJ, Doree C, Mathur A, Watt S, Martin-Rendon E. Stem cell treatment for acute myocardial infarction. *Cochrane Database Syst Rev*. 2012;2:CD006536.
  132. Kang L, Wang J, Zhang Y, Kou Z, Gao S. iPS cells can support full-term development of tetraploid blastocyst-complemented embryos. *Cell Stem Cell*. 2009;5:135–138.
  133. Zhao XY, Li W, Lv Z, Liu L, Tong M, Hai T, Hao J, Guo CL, Ma QW, Wang L, Zeng F, Zhou Q. Ips cells produce viable mice through tetraploid complementation. *Nature*. 2009;461(7260):86–90. doi: 10.1038/nature08267.
  134. Okita K, Ichisaka T, Yamanaka S. Generation of germline-competent induced pluripotent stem cells. *Nature*. 2007;448:313–317.
  135. Ohi Y, Qin H, Hong C, et al. Incomplete DNA methylation underlies a transcriptional memory of somatic cells in human iPS cells. *Nat Cell Biol*. 2011;13:541–549.
  136. Chin MH, Mason MJ, Xie W, et al. Induced pluripotent stem cells and embryonic stem cells are distinguished by gene expression signatures. *Cell Stem Cell*. 2009;5:111–123.
  137. Lister R, Pelizzola M, Kida YS, et al. Hotspots of aberrant epigenomic reprogramming in human induced pluripotent stem cells. *Nature*. 2011;471:68–73.
  138. Bar-Nur O, Russ HA, Efrat S, Benvenisty N. Epigenetic memory and preferential lineage-specific differentiation in induced pluripotent stem cells derived from human pancreatic islet beta cells. *Cell Stem Cell*. 2011;9:17–23.
  139. Marchetto MC, Yeo GW, Kainohana O, Marsala M, Gage FH, Muotri AR. Transcriptional signature and memory retention of human-induced pluripotent stem cells. *PLoS One*. 2009;4:e7076.
  140. Kim K, Zhao R, Doi A, Ng K, Unternaehrer J, Cahan P, Huo H, Loh YH, Aryee MJ, Lensch MW, Li H, Collins JJ, Feinberg AP, Daley GQ. Donor cell type can influence the epigenome and differentiation potential of human induced pluripotent stem cells. *Nat Biotechnol*. 2011;29:1117–1119.
  141. Polo JM, Liu S, Figueroa ME, Kulalert W, Eminli S, Tan KY, Apostolou E, Stadtfeld M, Li Y, Shioda T, Natesan S, Wagers AJ, Melnick A, Evans T, Hochedlinger K. Cell type of origin influences the molecular and functional properties of mouse induced pluripotent stem cells. *Nat Biotechnol*. 2010;28:848–855.
  142. Feng Q, Lu SJ, Klimanskaya I, Gomes I, Kim D, Chung Y, Honig GR, Kim KS, Lanza R. Hemangioblastic derivatives from human induced pluripotent stem cells exhibit limited expansion and early senescence. *Stem Cells*. 2010;28:704–712.
  143. He JQ, Ma Y, Lee Y, Thomson JA, Kamp TJ. Human embryonic stem cells develop into multiple types of cardiac myocytes: action potential characterization. *Circ Res*. 2003;93:32–39.
  144. Gupta MK, Illich DJ, Gaarz A, Matzkies M, Nguemo F, Pfannkuche K, Liang H, Classen S, Reppel M, Schultze JL, Hescheler J, Sarić T. Global transcriptional profiles of beating clusters derived from human induced pluripotent stem cells and embryonic stem cells are highly similar. *BMC Dev Biol*. 2010;10:98.
  145. van Laake LW, Qian L, Cheng P, Huang Y, Hsiao EC, Conklin BR, Srivastava D. Reporter-based isolation of induced pluripotent stem cell- and embryonic stem cell-derived cardiac progenitors reveals limited gene expression variance. *Circ Res*. 2010;107:340–347.
  146. Cao F, Wagner RA, Wilson KD, Xie X, Fu JD, Drukker M, Lee A, Li RA, Gambhir SS, Weissman IL, Robbins RC, Wu JC. Transcriptional and functional profiling of human embryonic stem cell-derived cardiomyocytes. *PLoS One*. 2008;3:e3474.

## Induced Pluripotent Stem Cells for Post–Myocardial Infarction Repair: Remarkable Opportunities and Challenges

Pratik A. Lalit, Derek J. Hei, Amish N. Raval and Timothy J. Kamp

*Circ Res.* 2014;114:1328-1345

doi: 10.1161/CIRCRESAHA.114.300556

*Circulation Research* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231

Copyright © 2014 American Heart Association, Inc. All rights reserved.

Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:

<http://circres.ahajournals.org/content/114/8/1328>

**Permissions:** Requests for permissions to reproduce figures, tables, or portions of articles originally published in *Circulation Research* can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the [Permissions and Rights Question and Answer](#) document.

**Reprints:** Information about reprints can be found online at:  
<http://www.lww.com/reprints>

**Subscriptions:** Information about subscribing to *Circulation Research* is online at:  
<http://circres.ahajournals.org/subscriptions/>