Title
Embryonic Stem Cell–Derived Cardiac Myocytes Are Not Ready for Human Trials

Journal
Circulation Research, 115(3)

ISSN
0009-7330

Authors
Anderson, Mark E
Goldhaber, Joshua
Houser, Steven R
et al.

Publication Date
2014-07-18

DOI
10.1161/circresaha.114.304616

Peer reviewed

Published in final edited form as:
Circ Res. 2014 July 18; 115(3): 335–338. doi:10.1161/CIRCRESAHA.114.304616.

Embryonic Stem Cell-Derived Cardiac Myocytes Are Not Ready For Human Trials

Mark E Anderson[1], **Joshua Goldhaber**[2], **Steven R Houser**[3], **Michel Puceat**[4], and **Mark A Sussman**[5,*]

[1]Departments of Internal Medicine and Molecular Physiology & Biophysics, Cardiovascular Research Center, Carver College of Medicine, University of Iowa, Iowa City, IA 52242-1081

[2]Cedars-Sinai Heart Institute, Los Angeles, CA 90048

[3]Department of Physiology and Cardiovascular Research Center, Temple University School of Medicine, Philadelphia, PA 19034

[4]INSERM UMR 910, GMGF, Université Aix Marseille, TeamPhysiopathology of cardiac development, Faculté de Medecine La Timone 13885 Marseille, France

[5]Biology Department, Integrated Regenerative Research Institute, San Diego State University, San Diego, CA 92182

Brief summary

Recently a preclinical report assessed the efficacy, safety, and feasibility of using human embryonic stem cells to regenerate infarcted myocardium in a non-human primate model. This commentary evaluates that study by pointing out key weaknesses, offering an alternative perspective, and summarizing major unresolved issues. Our conclusion is that significant challenges remain before human embryonic stem cells are ready for use in clinical trials.

Ischemic heart disease (IHD) affects millions of patients. The cardiac dysfunction that results from IHD is secondary to the death of cardiac muscle cells. Current IHD treatments are inadequate and there is a need to develop novel therapies to improve the lives of affected patients. New cellular therapies are being developed for repairing damaged hearts via induction of cardiac myocyte regeneration. In our view, approaches that can regenerate new, functional, myocardium need to be tested first in small animal models and then in appropriate large animal models. Those approaches that are shown to be safe and to improve cardiac function in preclinical studies should then be considered for clinical application.

In a recent report in *Nature*, Chong and collaborators describe experiments designed to test the potential of human embryonic stem cell-derived cardiac myocytes (hESC-CMs) to regenerate infarcted myocardium in a non-human primate model.[1] This study was proposed by the authors as proof of concept for trials of these cells in patients. Our commentary looks at this report with an eye toward safety and efficacy of hESC-CMs.

Correspondence to: Mark A Sussman, Biology Department, Integrated Regenerative Research Institute, San Diego State University, San Diego, CA 92182, heartman4ever@icloud.com.

*All authors contributed equally and are listed alphabetically

A major advance in the study of Chong et al. is that an approach has been developed to produce large quantities of hESC-CMs that appear to be sufficient for future use in humans with IHD. The authors were able to amplify one billion hESC-CMs, a number purported sufficient to regenerate an adult human infarcted heart. This approach addresses and potentially solves issues related to scaling up the production of hESC-CMs, which has been previously described by others using suspension culture methods for mouse and human ESC-CMs. [2–4] However, because of the limited analysis of the biological and functional effects of cryopreserved cells (see extended data Figure 1), additional characterizations would be required to substantiate the claim that cryopreserved cells are truly safe and effective for the *in vivo* studies.

The remainder of the study was designed to test if these cells were safe and efficacious in a more clinically relevant large animal, nonhuman primate model. State-of-the-art technologies (i.e., cells engineered to express the Ca^{2+} indicator GCaMP3 as previously described[5]) were used to evaluate the electrical coupling of hESC-CMs. Gene targeting for GCaMP3, however, was performed using the zinc finger nuclease methodology, which is known to exhibit off target effects that were not adequately addressed. Clinical implementation would require characterization of potential off target effects, which typically requires whole genome sequencing that reduces the clinical applicability of the study. The results presented suggest at least partial coupling of injected cells with the endogenous myocardium. However, the evidence provided of sarcomere formation or intercalated disks is debatable in GFP-defined areas, some of which appear more reminiscent of scar tissue (Figure 1, IF panels g to i). Unfortunately, all primates receiving hESC-CMs developed arrhythmias, while untreated controls were arrhythmia free. No significant reduction in infarct size was demonstrated (infarct size was 7.3% and 10.4% of the left ventricle in 2 control hearts and ranged from 3.7% to 9.5% in 4 cell-grafted hearts, P=NS).

Despite the substantial efforts put forth by the authors (dubbed “mending the broken heart” by the lay press and senior author), this study provides only anecdotal data in support of their primary hypothesis because of the small number of animals studied (1–2 monkeys at each time point), small infarcts, and lack of studies of pump function or electrical properties. The experiments presented provide strong evidence that hESC-CMs are not safe (arrhythmias in every animal) but fail to provide evidence that they improve cardiac function or regenerate myocytes. In addition, the use of ESC-CMs necessitates life-long immunosuppression to prevent cell rejection, which severely undermines the clinical feasibility and desirability of this approach.

The Murry group reported in a 2012 Nature paper[6] that hESC-CMs suppress ventricular arrhythmias in a small animal model. In this study, they now show that as the number of hESC-CMs is scaled up and heart size increases, arrhythmias are induced. This is very confusing to the scientific community. Although puzzling, there was significant hope after the Shiba et al. paper was published[6] that hESC-CMs somehow would work better than most clinically available anti-arrhythmic drugs in suppressing ventricular arrhythmias. However, the data in the current study by Chong et al. strongly suggest that the arrhythmia risk of hESC-CMs increases with the number of cells injected and decreases with the resting heart rate. The data presented by Chong et al. clearly document that the number of hESC-

CM-induced arrhythmias was predictable from the mass of cells that survive within the damaged monkey heart. Because they injected *one billion* cells, numerous side effects occur including arrhythmias that are presumably triggered because cells couple poorly to the parent myocardium. The larger the mass of injected cells, the greater the areas of poor coupling that will be present, raising arrhythmia risk. The authors state that more cells are going to be necessary for larger human hearts after MI; this will further increase the arrhythmias risk.

The authors suggest that there is an inverse relationship between the resting heart rate of an animal and the arrhythmia risk after injection of hESC-CMs. Given that heart rates in humans are significantly slower than in these monkeys, the arrhythmia risk should be increased. Three-fourths of cell-treated animals had arrhythmias during their last recording prior to sacrifice, mostly within 2 weeks of cell therapy; this is inconsistent with the authors' claims in the abstract and throughout the body of the report that arrhythmias subside over time. How could the authors possibly know if the arrhythmias would have actually "resolved" over time? How can they possibly know that the animals were not in distress or conscious if these arrhythmias were only recorded on telemetry?

In our view, a major conclusion of this study is that the arrhythmia risk of hESC-CMs will increase in humans. These results show that hESC-CMs have a high potential to be dangerous to patients, at least in the dosage that is being proposed by the investigators.

An important goal of cell therapy for IHD is that cells couple with host myocardium and have normal contractile properties. The data shown by Chong et al. in Figure 4h document that the engrafted hESC-CMs do not have normal function. A Ca^{2+} alternans behavior, predictive of arrhythmia risk, is shown. This type of abnormal Ca^{2+} regulation has been clearly linked to lethal arrhythmias.[7]

The mechanism of the arrhythmias induced by hESC-CMs in the report by Chong et al cannot be determined from the limited data provided. However, the arrhythmias observed in this study could have several origins. One potential cause is triggering from abnormal intracellular Ca^{2+} handling, potentially consistent with the observed Ca^{2+} alternans. The observed abnormalities in intracellular Ca^{2+} handling suggest that the electrophysiological properties and Ca^{2+} transients of hESC-CMs are significantly different; this will produce regional variation in repolarization, a known precursor of arrhythmias. The report by Chong et al also does not address these issues adequately. The limited images of Ca^{2+} transients do suggest at least partial coupling of injected hESC-CMs and host myocardium. However, the data clearly show fundamental differences in Ca^{2+} transients between hESC-CMs and host myocardium. These will be likely hot spots for arrhythmias. *In vivo* evidence of contraction might be ephaptic conduction from host myocardium and not true electro-mechanical coupling.

Another potential cause of arrhythmias is the presence of a prominent non-cardiac cell population. Cell heterogeneity is likely to be an issue in the study by Chong et al. One can obtain a highly enriched single cell lineage from hESC-CMs without sorting the cells at any step; in the work of Chong et al., the yield of cardiomyocytes was assessed by TnT FACS.

We know the pitfalls of this approach using anti TnT antibodies.[8] High content imaging would have helped to assess the percentage of contaminating cells. The observation of beating cells or monolayers in a dish does not mean that 100% of cells are cardiac cells. In the current study, there are no images of cells that have been grafted. This lack of clarity about the purity of the engrafted cell population creates uncertainty regarding the interpretation of the published data. Another and non-mutually exclusive explanation for the observed arrhythmias is that the large number of cells injected produced areas where coupling of hESC-CMs and parent myocardium was poor.

The take home message from this study is that hESC-CMs produce arrhythmias in small monkey hearts, which suggests that a greater arrhythmia risk will be present in larger human hearts.

The Puceat laboratory carried out similar studies in 2008 using small Rhesus macaque infarcted hearts injected with sorted Rhesus ES cells-derived and highly enriched mesodermal cardiogenic cells which differentiated into cardiomyocytes. Arrhythmias were never observed in any of 8 grafted primates even 2 or 3 months post-graft.[9] Collectively, the observations of Chong et al. and Puceat et al. suggest that the number and type of injected cells, hESC-CMs versus ES cell-derived mesodermal cells in the course of cardiac differentiation explain the differences in arrhythmia burden between these studies.

Chong et al. claim that their approach produces “remuscularization” of the damaged heart. Remuscularization implies that the new myocytes are permanently integrated into the host heart and that they make a functional contribution to the ventricle. The study by Chong et al. was not properly designed to address this issue. Although a GFP signal was detectable in some monkeys, it remains unclear from the data provided how many (if any) of the 1 billion cells survived in the different animals studied. Data from other groups suggest that by 1 month, <1% of cells survive.[10] Because so many cells (*1 billion*) were injected, it took longer for the cells to die off. Hence the most impressive histology presented in the paper was shown relatively early at day 14 (Figure 1). Importantly, histology is not an ideal approach to address the fraction of hESC-CMs that survived in each of the monkeys used in the study by Chong et al. Techniques that can reliably measure the number of myocytes surviving after injection into damaged hearts have been validated and should be considered in the future.

The purpose of cell therapy is to improve myocardial function. Regeneration of myocardium, in itself, is not meaningful if it does not translate into improved contractile performance. The report by Chong et al. was unable to show any functional benefit of hESC-CMs. This, in part, resulted from the fact that the infarcts were small; based on Table 1, the “40%” is really only 2.1% of left ventricular mass (1% if outlier P5 is excluded). Readers should also consider that the GFP expressing cells that were used to calculate engraftment were not likely all cardiomyocytes, and other, non-myocardial, cell types were not investigated. Conclusions based exclusively on histology should always be made cautiously. In this regard, the muscle fiber orientation of the putative hESC-CM-derived myocardium looks like endogenous, not regenerated, heart muscle and should be validated by alternative techniques. It is also surprising that the pro-survival cocktails that the same

group has reported to significantly enhance survival of hESC-CMs[11] was not employed to enhance cell survival, rather than having to inject 1 billion cells.

The study by Chong et al. also suffers from a number of design problems that reduce the potential impact of the work. Indeed, studies in small primates have numerous limitations. For example, it is difficult to assess the ability of cell therapy to improve cardiac function when the infarction is small and has such modest effects on ventricular function. Models that fail to recapitulate the depressed cardiac function of patients that might benefit from these therapies have little value. Another major problem with the study by Chong et al. is that too few primates were used, the duration of follow-up was inconsistent (from 2 weeks onward), and the primates were of dramatically different ages (5–14 years), with missing sham data at certain time points. Overall, the study is preliminary and the data anecdotal. The study is statistically underpowered to provide useful information about potential mechanical benefits of hESC-CM therapy, but adequately powered to show that arrhythmias were induced in every animal.

In our view, preclinical cell therapy experiments need to be performed in large animal models of cardiac injury with characteristics that closely resemble critical aspects of disease burden in humans. There can be a definite role for using non-human primates in certain circumstances, but for obvious reasons the bar must be set high. To us, the use of non-human primates in the study by Chong et al. was not adequately justified. The primate weights were 8–10 kg. Therefore, heart size was about 8x smaller than the normal adult human heart. The resting heart rate of these monkeys was significantly greater than in humans. Chong et al. argue that humans will require greater numbers of injected hESC-CMs and that this will enhance arrhythmias. Pig and sheep weighing 50–70 kg have hearts sizes and heart rates more similar to humans. Furthermore, larger MIs can be induced in these models[12–14], and these models are significantly less expensive and logistically cumbersome than primates. The study by Chong et al., together with the prior report by the Puceat group[9], confirms that primates are not the best available model to test the potential of pluripotent stem cell-derived cardiac derivatives to restore failing ventricular function.

Another very serious problem with the study by Chong et al is that the teratoma risk of ESC-based cell therapy is understated. The studies were performed in a small number of monkeys and for a short time period; therefore, the proclivity of ESC-CMs to produce malignancies was not evaluated. The authors suggest that human trials could require up to 8 billion hESC-CMs; those cells that survive would be in the patients for the remainder of their life. In our view, it is likely that a few of these cells will be pluripotent. Even if the occurrence of teratomas was very rare, it would still prohibit the use of these cells as a clinical therapy. While the arrhythmia risk of hESC-CMs might be managed with ICDs, the risk of malignancies could make this type of cells unsafe for use in humans.

There is a clear need to develop cell therapies that can repair the damaged heart. Given that considerable work has already been performed with a variety of cell types, it is important that new cells be shown to offer advantages over those tested heretofore. Various non-ESC-derived cells have been used by several groups in recent years, with encouraging results in terms of both safety and efficacy.[15–17] In contrast, the study by Chong et al shows that

significant challenges must be overcome before hESC-CMs are ready for early stage clinical trials. The most significant problems are that ES-derived cells have known teratoma risks, require long-term immunosuppression, may cause sudden death, and it is unclear that they improve cardiac function.

References

1. Chong JJ, Yang X, Don CW, Minami E, Liu YW, Weyers JJ, Mahoney WM, Van Biber B, Palpant NJ, Gantz JA, Fugate JA, Muskheli V, Gough GM, Vogel KW, Astley CA, Hotchkiss CE, Baldessari A, Pabon L, Reinecke H, Gill EA, Nelson V, Kiem HP, Laflamme MA, Murry CE. Human embryonic-stem-cell-derived cardiomyocytes regenerate non-human primate hearts. Nature. 2014 Apr 30. (e-pub ahead of print).
2. Shafa M, Krawetz R, Zhang Y, Rattner JB, Godollei A, Duff HJ, Rancourt DE. Impact of stirred suspension bioreactor culture on the differentiation of murine embryonic stem cells into cardiomyocytes. BMC Cell Biol. 2011; 12:53. [PubMed: 22168552]
3. Niebruegge S, Nehring A, Bär H, Schroeder M, Zweigerdt R, Lehmann J. Cardiomyocyte production in mass suspension culture: embryonic stem cells as a source for great amounts of functional cardiomyocytes. Tissue Eng Part A. 2008; 14:1591–601. [PubMed: 18593268]
4. Lecina M, Ting S, Choo A, Reuveny S, Oh S. Scalable platform for human embryonic stem cell differentiation to cardiomyocytes in suspended microcarrier cultures. Tissue Eng Part C Methods. 2010; 16:1609–19. [PubMed: 20590381]
5. Tallini YN, et al. Imaging cellular signals in the heart in vivo: cardiac expression of the high-signal Ca21 indicator GCaMP2. Proc Natl Acad Sci USA. 2006; 103:4753–4758. [PubMed: 16537386]
6. Shiba Y1, Fernandes S, Zhu WZ, Filice D, Muskheli V, Kim J, Palpant NJ, Gantz J, Moyes KW, Reinecke H, Van Biber B, Dardas T, Mignone JL, Izawa A, Hanna R, Viswanathan M, Gold JD, Kotlikoff MI, Sarvazyan N, Kay MW, Murry CE, Laflamme MA. Human ES-cell-derived cardiomyocytes electrically couple and suppress arrhythmias in injured hearts. Nature. 2012; 489:322–5. [PubMed: 22864415]
7. Clusin WT. Mechanisms of calcium transient and action potential alternans in cardiac cells and tissues (review). Am J Physiol - Heart and Circ Physiol. 2008; 294:H1–H10. [PubMed: 17951365]
8. Puceat M. Could a pluripotent stem cell give rise to a high yield of a single cell lineage: a myocardial cell? Curr Opin Genet Dev. 2013; 23:498–499. [PubMed: 23850377]
9. Blin G, Nury D, Stefanovic S, Neri T, Guillevic O, Brinon B, Bellamy V, Rücker-Martin C, Barbry P, Bel A, Bruneval P, Cowan C, Pouly J, Mitalipov S, Gouadon E, Binder P, Hagège A, Desnos M, Renaud JF, Menasché P, Pucéat M. A purified population of multipotent cardiovascular progenitors derived from primate pluripotent stem cells engrafts in postmyocardial infarcted nonhuman primates. J Clin Invest. 2010; 120:1125–1139. [PubMed: 20335662]
10. Templin C, Zweigerdt R, Schwanke K, Olmer R, Ghadri JR, Emmert MY, Müller E, Küest SM, Cohrs S, Schibli R, Kronen P, Hilbe M, Reinisch A, Strunk D, Haverich A, Hoerstrup S, Lüscher TF, Kaufmann PA, Landmesser U, Martin U. 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. [PubMed: 22767659]
11. Buxton DB, Antman M, Danthi N, Dilsizian V, Fayad ZA, Garcia MJ, Jaff MR, Klimas M, Libby P, Nahrendorf M, Sinusas AJ, Wickline SA, Wu JC, Bonow RO, Weissleder R. Report of the National Heart, Lung, and Blood Institute working group on the translation of cardiovascular molecular imaging. Circulation. 2011; 123:2157–63. [PubMed: 21576680]
12. Laflamme MA, Chen KY, Naumova AV, Muskheli V, Fugate JA, Dupras SK, Reinecke H, Xu C, Hassanipour M, Police S, O'Sullivan C, Collins L, Chen Y, Minami E, Gill EA, Ueno S, Yuan C, Gold J, Murry CE. Cardiomyocytes derived from human embryonic stem cells in pro-survival factors enhance function of infarcted rat hearts. Nat Biotechnol. 2007; 25:1015–24. [PubMed: 17721512]

13. Ménard C, Hagège 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. [PubMed: 16168783]

14. 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. [PubMed: 23371930]

15. Williams AR, Hare JM. Mesenchymal stem cells: biology, pathophysiology, translational findings, and therapeutic implications for cardiac disease. Circ Res. 2011; 109:923–40. [PubMed: 21960725]

16. Telukuntla KS, Suncion VY, Schulman IH, Hare JM. The advancing field of cell-based therapy: insights and lessons from clinical trials. J Am Heart Assoc. 2013; 2:e000338. [PubMed: 24113326]

17. Kovacic JC, Harvey RP, Dimmeler S. Cardiovascular regenerative medicine: digging in for the long haul. Cell Stem Cell. 2007; 1:628–33. [PubMed: 18371404]