A big heart

By Tracey Baas, Senior Editor

Cell therapies in the clinic for heart failure draw from divergent sources of nonembryonic stem cells but have shown only minimal improvements in cardiac function. A **University of Washington** team thinks human embryonic stem cell–derived cardiomyocytes may do a better job and has used the cells to remuscularize infarcted monkey hearts.[1]

Larger preclinical studies, using more consistent methods, will need to address the arrhythmia seen in the study and show conclusively that the cells improve cardiac function.

At least 11 companies have stem cell therapies in Phase II testing or earlier to treat myocardial infarction (MI). The clinical-stage products use either autologous or allogeneic adult stem cells. Those cells are rare in mature tissues, and as a result isolation and expansion is a challenge.

Mesenchymal stem cells (MSCs) are a more plentiful source of adult stem cells and can differentiate into osteoblasts, chondrocytes and adipocytes. *Ex vivo*, MSCs can be induced to differentiate into cardiomyocytes. However, the flexibility in their differentiation capacity could be a drawback, as MSCs could result in unwanted cell types forming in cardiac tissue.

Alternatively, human embryonic stem cells (hESCs) can be grown easily in culture and can differentiate into a single cell type, such as cardiomyocytes. The problem is quantity—protocols to provide large-scale quantities for cardiac repair need to be optimized.

The University of Washington team previously transplanted about 10^6, 10^7 and 10^8 hESC-cardiomyocytes (CMs) that were cultured and directly transferred to mouse, rat and guinea pig models of MI.[2–5] For a nonhuman primate model, the researchers estimated that about 10^9 cells would be required for a 50 g macaque heart, which was a big hurdle technically. These numbers would be close to what is required for a 300 g human heart.

To produce enough cells for the nonhuman primate model, the group differentiated hESCs into cardiomyocytes. The resulting cells spontaneously beat in culture. Rather than transferring the cells directly into an animal after culture, the cells were treated with a prosurvival cocktail and then were cryopreserved.

Two weeks before hESC-CM delivery, pigtail macaques underwent myocardial ischemia/reperfusion to generate infarcts. Five days before cell delivery, they were put on an immunosuppressive regimen to prevent graft rejection.

Four animals received cells via epicardial puncture sites that were stabilized with sutures. Two animals underwent the same procedure with vehicle.

The treated monkeys showed remuscularization of the infarct area with grafts averaging about 40% of the infarct mass. From week 2 to week 12, the engrafted cells progressively matured, as shown by increased myofibril alignment, sarcomere registration and cardiomyocyte diameter.

Because of the small number of animals used, the team could not conclude whether the procedure significantly improved cardiac function. Two of the four treated animals showed increases in ejection fraction, whereas the other two did not.

Overall, the hESC-CM grafts showed infiltration of lymphocytes and perfusion of blood vessels. The grafts were visible by imaging and showed electromechanical coupling to macaque hearts.

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> ***—Eduardo Marbán, Cedars-Sinai Heart Institute***

Continuous electrocardiogram recording showed that the four treated monkeys had periods of arrhythmia during the first two weeks of recovery. The authors wrote that further studies are needed to tease out the underlying mechanisms that contributed to arrhythmia.

Results were published in *Nature*. The authors did not return requests for comment.

Eduardo Marbán, director of the **Cedars-Sinai Heart Institute**, noted that because the researchers were focused on showing the feasibility of the approach, "the protocol varied significantly from animal to animal. An important next step would be to adhere to a single experimental protocol in a rigorous manner that would enable statistical analysis once all the data are collected."

Marbán also was the principal investigator on the NIH-funded Phase I CADUCEUS (CArdiosphere-Derived aUtologous stem CElls to reverse ventricULar dySfunction) trial of cardiosphere-derived autologous stem cells for heart regeneration after MI. His technology, licensed by **Capricor Therapeutics Inc.**, forms the basis for an ongoing Phase I/II trial, ALLSTAR (ALLogeneic heart Stem cells To Achieve myocardial Regeneration), which uses allogeneic, cardiosphere-derived stem cells for the same indication.

In the CADUCEUS trial, 17 patients received the cell-based therapy and had no cardiac tumors or major adverse cardiac events. Patients showed a decrease in cardiac scar mass and an increase in viable heart mass and contractility; no such changes were seen in eight patients randomly assigned to receive standard care. But there were no substantial differences in left ventricular ejection fraction between the two groups.

Early steps

The researchers now need to generate more evidence of increased cardiac function, show that arrhythmias can be circumvented or are not problematic and rule out graft rejection and teratoma formation.

Roberto Bolli, director of the Division of Cardiology and the Institute of Molecular Cardiology and vice chair for research in the Department of Medicine at the **University of Louisville**, said that the study authors were jumping the gun with their conclusions that "large hESC-CM grafts are successfully perfused by host vasculature and are viable long term."

"The results are preliminary and anecdotal. Four monkeys were given cells. For the researchers to conclude that their data show that the grafts 'are viable long term' is extremely premature," he said. "With a follow-up of 2 weeks for 1 monkey, 4 weeks for 2 monkeys and 12 weeks for 1 monkey, no such conclusion is possible."

Bolli wants to see larger and more detailed studies, with a longer follow-up, that show functional relevance and safety. "We do not know whether transplantation of these cells improved cardiac function or the structure of the heart, which are both major goals of cell therapy," he said. Thus, Bolli is not convinced that the embryonic cells are an advance over the adult stem cell–derived products in the clinic.

Bolli said that he was still concerned about the potential of ESCs to form tumors and induce graft rejection. In contrast, he said, "to date, not one single major adverse effect has been reported that could be ascribed to transplantation of adult stem cells in patients."

The key safety issue thus far is arrhythmias. Moreover, humans have larger and slower hearts than macaques—two factors that correlate with increased risk of developing arrhythmias after cell engraftment.[6–8]

Mary Wagner, an assistant professor of pediatrics and director of the Center for Cardiovascular Biology at the **Emory University School of Medicine**, said that mapping the electrical activity of the heart would be a good way to evaluate if arrhythmias are caused by the transplanted hESC-CMs or represent a more general response to the procedure. "For example, 3D mapping techniques for quantifying the propagation of electrical activity could map the arrhythmias and lead to a better understanding of whether cellular engraftment contributes to the side effect," she said.

"A lack of gap junctions early after transplantation in immature cardiomyocytes may produce poor coupling between the host and graft and can result in slow or intermittent conduction and possibly arrhythmia," said Chunhui Xu, an associate professor of pediatrics and director of the Cardiomyocyte Stem Cell Laboratory at the Emory University School of Medicine. "In the *Nature* study, at 14 days post-transplantation, there is little evidence of gap junction protein α1, 43 kDa (GJA1; CX43; connexin-43)—suggesting cardiomyocyte immaturity—but [the protein] is seen 12 weeks post-transplantation."

Xu said that techniques that speed up maturation of engrafted cardiomyocytes could help prevent arrhythmias.

"An alternative strategy could be to introduce earlier cardiomyocytes, or cardiac progenitors, before they have begun to autonomously beat," said Emile Nuwaysir, COO and VP of R&D, manufacturing and quality systems at **Cellular Dynamics International Inc.** "This may work because the cells, as they mature *in vivo*, might listen to the endogenous pacemaker as they become electromechanically active and would have no adaptation period after injection."

Markus Krane, head of the Department of Experimental Surgery at the **German Heart Centre Munich**, said that the cell therapy could benefit from a different delivery approach. "In an aged person with a chronically failing heart and increased epicardial fat, simple repetitive cardiac puncture would be a difficult clinical scenario," he said. "In this setting, it would be more advantageous to deliver small, preformed hESC-CM-based cell clusters or tissue grafts for implantation under the epicardial fat layer."

Philippe Menasche liked the idea of optimizing stem cell delivery by a pericardial flap. Menasche is a professor of thoracic and cardiovascular surgery at the **University Paris Descartes**, chief of the Heart Failure Surgery Unit at **Georges Pompidou European Hospital** and director of an **Institut National de la Santé et de la Recherche Médicale** (INSERM) laboratory focused on cell therapy for cardiovascular diseases.

Menasche also is a co-investigator on a Phase I trial at **Paris Public Hospital**. The ESCORT (transplantation of human Embryonic Stem Cell–derived prOgenitors in severe heaRT failure) trial is recruiting patients.

"The pericardial flap is considered a growth factor–rich tissue, so using it to cover hESC-CMs or a scaffold seeded with hESC-CMs could potentially provide the cells with a natural, tissue-based, prosurvival environment," he said.

"By using a contractile patch made from hESC-CMs or human induced pluripotent stem cell–derived CMs—potentially sutured over the most damaged cardiac region—this would provide a way to enhance the pumping function of the damaged cardiac tissue. The transplanted patch could also be insulated from the host's own cardiac cells," added Jonathan Epstein. "This insulation could block the arrhythmia that is induced by competing electromechanical coupling of two sets of cardiomyocytes—self and nonself."

Epstein is chair of the Department of Cell and Developmental Biology and scientific director of the Penn Cardiovascular Institute at the **Perelman School of Medicine at the University of Pennsylvania**.

The patent and licensing status of the University of Washington's findings was not available at publication time.

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COMPANIES AND INSTITUTIONS MENTIONED

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