JOURNAL OF THE AMERICAN COLLEGE OF CARDIOLOGY
VOL. 71, NO. 4, 2018

PUBLISHED BY ELSEVIER

EDITORIAL COMMENT

Trial of Embryonic Stem Cell–Derived Cardiac Progenitor Cells

An Encouraging Start*

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The rapidly growing number of people worldwide with debilitating chronic diseases (1) has led to significant research into the fast expanding field of regenerative medicine (2), with the hope of not just temporizing disease progression or palliating symptoms, but also inducing very meaningful improvement by repairing organ and/or tissue damage and regenerating new functional tissue. Cardiovascular disease is the leading cause of death in the world, and the heart has logically been the focus of the largest amount of basic and clinical research in this field, which has been dominated by the use of various types of stem cells.

It was long thought that the heart was terminally differentiated, with a fixed number of cardiomyocytes at birth, and therefore incapable of any regeneration (3). However, the heart undergoes an estimated 2% to 4% loss of cells per year as a result of programmed cell death, thus requiring the same number to be regenerated annually to maintain normal function. This property suggests an intrinsic capacity of the heart to regenerate. The question of cardiomyocyte renewal has been the subject of a recent consensus statement from the American Heart Association (4). These experts suggest that most of the cell turnover reported, especially in response to injury, is not turnover of cardiomyocytes, but of supporting cells such as fibroblasts, smooth muscle cells, and endothelial cells. However, this regenerative capacity is very inefficient, especially compared with that of other organs such as the liver. This problem is compounded by the body's natural response to injury of fibrosis and scarring, rather than proliferation, to avoid development of malignancy.

There are only 2 sources of human stem cells, embryonic and postnatal, typically adult origin. Until very recently, all clinical trials have been conducted using autologous adult stem cells, primarily from bone marrow, to avoid the expected adverse alloimmune response and the need for multidrug immunosuppression expected from the administration of foreign cells. Several meta-analyses (5,6) demonstrated a statistically significant benefit with the use of several types of adult stem cells, primarily bone marrow derived, but the clinical benefit of these cells was less than anticipated.

Possible explanations for this modest response with the use of autologous cell sources include the progressive senescence of stem cells with age (7), compounded by added negative effects in patients with chronic disease, including further reduction in function and absolute number (8). Newer strategies for use of autologous bone marrow cells include triage of potential candidates in clinical trials (e.g., phase III Cardi-AMP Heart Failure Trial; NCT02438306) by requiring a minimum number of endothelial progenitor cells in the bone marrow for enrollment, to study those candidates with the greatest chance of improvement.

The recognition of the unique lack of immunogenic antigens on the surface of mesenchymal-type stem cells, regardless of source, has led to a progressive exploration of the clinical use of these allogeneic cells (9). This relative immune privilege allows use of ideal young donors and repeated passaging of these donor cells to provide a single donor for hundreds of subjects enrolled in a clinical trial, such as the current phase III mesoblast trial for heart failure (Efficacy and

*Editorials published in the *Journal of the American College of Cardiology* reflect the views of the author and do not necessarily represent the views of *JACC* or the American College of Cardiology.

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ISSN 0735-1097/$36.00
https://doi.org/10.1016/j.jacc.2017.11.042

Safety of Allogeneic Mesenchymal Precursor Cells (Rexlemestrocel-L) for the Treatment of Heart Failure [DREAM HF-1]; NCT02032004) now nearing complete enrollment, with no evidence of significant measurable allospecific antibody formation without the use of immunosuppression. These observations have also led to a growing interest in the use of umbilical cord (10) and other perinatal tissue (11) as sources of stem cells, with increasing documentation of the similar lack of immunogenicity of these cells when obtained immediately postpartum.

Given the lack of demonstration of true differentiation of any transplanted stem cells into functioning cardiomyocytes, the current consensus is that cell therapy exerts its benefit primarily by a paracrine mechanism (12). The demonstration of the presence of lineage-specific cardiac progenitor cells in the heart (13,14) has led to an increasing examination of the use of these cells as a potentially superior strategy over transplantation of other types of stem cells relying totally on paracrine stimulation of intrinsic mechanism for regeneration. In addition, there has been significant exploration of the use of pluripotent cells induced from skin fibroblasts and other sources (15).

The other major source of stem cells is from human embryos, which have the greatest pluripotency, or ability to derive into every cell type needed for generation of any organ and tissue in the body (16,17). However, the controversy and intensity of debate associated with use of embryonic stem cells (ESCs) have resulted in the destruction of many established cell lines and have limited the examination of their potential for clinical use.

The use of ESCs in preclinical animal studies confirmed the potent proliferative capacity and also the marked pluripotency of this type of cell, with resultant induction of multi-cell-type tumors called teratomas often in locations remote from the site of delivery. In addition, although prenatal in origin, these cells express a number of foreign epitopes on their surface that have required use of significant amounts of immunosuppression to prevent alloimmune-generated injury following delivery to the heart. There has also been concern about a proarrhythmic effect, although it has been shown that ESCs are unique in their ability to form gap junctions with native cardiomyocytes and generate integrated cardiac conduction (16). However, the demonstrated overall potency of these cells in improving cardiac function in animal models has led to ongoing interest in their potential clinical use for patients with heart failure.

Following extensive preclinical animal testing (17,18), as well as rigorous consideration of potential safety issues, in this issue of the *Journal*, Menasché et al. (19) report the use of ESC-derived cardiac progenitor cells for patients with ischemia-induced heart failure. The inclusion criteria for the study were as follows: age 18 to 81 years; documented prior myocardial infarction; stable New York Heart Association (NYHA) functional class III to IV heart failure symptoms with an ejection fraction (EF) of 15% to 35%; coronary anatomy suitable for surgical bypass grafting; pre-existing presence of an implantable cardioverter-defibrillator; and absence of measurable antibody to any of the donor antigens. Only 6 patients were enrolled over a 2-year period, largely because of the concomitant need for bypass surgery. The median follow-up was 12 months, with 1 patient only 6 months post surgery and cell delivery.

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All patients received 3-drug immunosuppression, including 240 mg of intravenous steroids given intraoperatively, and then a second dose the following day, with no long-term use thereafter, as well as daily doses of cyclosporine to maintain trough levels of 100 to 150 ng/ml and mycophenolate mofetil of 2 g/day. The latter 2 drugs were given for the first 2 months in the first 2 patients and were then reduced to only 1 month's duration in the last 2 patients. There were no reports of increased infection or nephrotoxicity with this regimen, and they remained responsive to third party allergens.

An early goal of this study was to prove the feasibility and scalability of generating this cell population. Menasché et al. (19) were able to derive a nearly pure population (97.5%) of clinical-grade cells by extensive surface marker screening tests, meeting this important milestone.

There are problems associated with the epicardial (and endocardial) injection method to deliver stem cells because of potential leakage from the injection site with each cardiac contraction. To avoid this problem, the ESC-derived cardiac progenitor cells in this study were first incorporated into a fibrin scaffold, as previously described by this group (18), and subsequently delivered at the completion of distal bypass graft anastomoses via a novel approach. A piece of the patient's native pericardium was cut to match the size of the cell-fibrin scaffold and was sutured around one-half of the infarct area. The cell scaffold was then inserted inside this pericardial pouch, in direct contact with the epicardium. The remaining one-half of the pericardial patch was then folded over and sutured to cover the other one-half of the infarct area, thereby creating a covered pouch.

The primary goal of the study was safety, with endpoints including the following: evidence of tumorigenicity, using fluorodeoxyglucose positron emission tomography and whole body computed tomography scanning for surveillance; proarrhythmia, as detected by serial examination of implantable cardioverter-defibrillators that had been placed pre-study; and donor-specific antibody formation using serial measurements with the Elispot assay (Mabtech, Cincinnati, Ohio). In addition, all subjects were followed for development of major adverse major cardiovascular events.

The study was remarkably free of evidence of any of the safety endpoints. Most reports of preclinical induction of teratoma formation in animal models occur within weeks to months of delivery of ESCs, but there were no tumors of any type noted by positron emission tomography scanning at 6 months or computed tomography scans at 12 months. There was also no evidence of proarrhythmia demonstrated over the follow-up period aside from 1 episode of asymptomatic 5-beat ventricular tachycardia. There was evidence of development of 3 clinically silent, but measurable titers of allospecific antibodies, 2 of which occurred within 10 days of cell delivery, and 1 that occurred 2 months later, which was directed at a different epitope, but also in low titer. All titers fell to undetectable levels by 4 months, and none were associated with clinical cardiac dysfunction. There were 2 deaths in the cohort, 1 perioperative death judged by external reviewers as unrelated to cell delivery and 1 at 22 months post-treatment of progressive heart failure, which was the only major adverse cardiovascular event reported in this cohort in follow-up. This late death also demonstrates a failure of this strategy to improve all subjects in the trial, a common finding in most clinical trials.

The secondary endpoints of the study included cardiac function assessed by change in EF and left ventricular volumes by echocardiography, as well as change in regional wall motion in areas of cell delivery. In addition, overall functional capacity was assessed by the 6-min walk test, Quality of Life Scale score, and self-assessed NYHA functional class. Given the very small number of subjects, there was no attempt at statistical comparisons, but there was a demonstrated improvement in all parameters assessed. Despite the inherent conundrum of segregating the net benefit of cell therapy on wall motion as a result of the concomitant placement of bypass grafts, including an internal mammary artery graft in all patients, Menasché et al. (19) demonstrated an improvement in wall motion in the area of cell delivery, an overall increase in EF, and a small reduction in left ventricular volumes. There was an average increase in the 6-min walk test result of 23 m and an average reduction in NYHA functional class from III to I/II in the 4 patients who reached the 1-year follow-up. The Quality of Life Scale scores showed comparable improvement.

Overall, the initial use of these ESC-derived cardiac progenitor cells seems to have met the primary endpoint of safety. The relatively short course of the 3-drug immunosuppression regimen used seems a potentially acceptable trade if the benefit can be corroborated in the absence of typical adverse clinical side effects. The use of additional agents such as antibodies directed at costimulatory molecules may enhance the suppression of alloantibody production and lower the net amount and duration of immunosuppression required. Unfortunately, there was no tagging or chromosomal difference to identify the number of transplanted cells retained in heart at 9 days from the 1 early death, but no signs of inflammation were evident.

The obvious major limitation of this study was the very small sample size, which allows only observational inferences to be made. The reporting of such a small cohort will likely be controversial, but the overall contribution to the knowledge in the field of this potent cell type was judged to warrant publication of these early results. Menasché et al. (19) justified stopping at this point, given the precedent in oncology trials of very small initial feasibility pilot trials with use of the more aggressive and potent new agents (20). Other shortcomings include surgical delivery, which may be applicable to a significant number of potential patients, but far fewer than could be treated by a catheter approach, which presumably is being explored.

The second rationale for stopping the study at this small number of subjects is the very rapid evolution of the field. This includes the increasing awareness of the equal potency and potential substitution of the transplanted cells by their secretome, referred to as exosomes or intracellular vesicles (21,22), as well as the expanding role of tissue engineering with the use of matrix and microRNA (23) alone, for a cell-free but cell-derived therapy. This cell-free strategy may prove to be not only the safest and easiest way to use these potent cells, but also potentially equally effective. If this strategy is proven safe, the field also needs to move to more routine examination of the use of multiple deliveries of cells or their products, or genes, to provide a potentially additive benefit with each additional administration, to maximize the net benefit (24).

Regenerative medicine remains one of the most promising strategies to provide not just palliation,

but also clinically meaningful improvement and recovery for patients with heart failure and potentially all forms of cardiovascular disease. This report adds another possible agent to the growing list of options.

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KEY WORDS alloreactivity, embryonic stem cells, heart failure, stem cells