Allogeneic Precursor Cells for Systolic Heart Failure
A Need for Mechanisms in Humans

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Systolic Heart Failure: Therapies Necessary
Congestive heart failure (CHF) is a major cause of cardiac morbidity and mortality. Loss of cardiomyocytes with replacement fibrosis is a common feature of all end-stage heart disease. Although data suggest that there is at least some regeneration of cardiomyocytes throughout life, it is clear that exogenous drug therapy and endogenous sources of regeneration and repair are insufficient to stop the progression of heart failure. The great need for improved therapies has stimulated the rapid translation of studies of stem and precursor cell therapy into clinical trials for heart failure and myocardial infarction. In this issue, Perin et al describe a phase II, dose escalation study of a mesenchymal stem cell (MSC) product for advanced heart failure.

MSCs: What Are They and What Do They Do?
MSCs are mesodermal-derived, multipotential, stromal cells found in several organs, most numerous in bone marrow and adipose tissue. They are defined by the ability to grow well in culture, differentiate into adipocytes, osteoblasts, and chondrocytes, and being positive for several surface markers, including CD90, CD105, HLA-DR, and CD271, whereas being negative for CD45 and MHCII. Mesenchymal progenitor cells (MPCs) are related, but also positive for surface markers CD31, CD105, Stro-1/3. Most MSC-like cells can also be differentiated in vitro into endothelial cells, smooth muscle cells, and fibroblasts.

MSCs were enthusiastically studied in vitro and showed therapeutic promise to (1) increase endothelial proliferation and vessel formation, (2) promote cardiomyocyte survival, and (3) differentiate into cardiomyocytes. In preclinical animal models, MSC/MPCs can increase endothelial and cardiomyocyte generation and preservation, improved infarct healing, and rescue of heart failure. Similar in vitro data were generated with other stem cells with mesenchymal origins, including cardiac- and bone marrow–derived (CD34+) stem cells. Spring boarding from these preclinical studies, many small human trials have been performed with MSCs, bone marrow mononuclear cells, c-kit+ cardiac stem cells, and cardiosphere-derived cells to regenerate cardiac cardiomyocytes and endothelium, as well as repair or regenerate injured and infarcted myocardium.

Despite varied cell product of use, timing, dose, delivery method, disease model, and protocol, many published studies have positive results. These studies consistently demonstrate a small improvement in ejection fraction or decrease in myocardial scar, less so functional improvement, and mortality benefit. There is a paucity of human data for increased endothelial density and de novo cardiomyocyte generation to explain these possible effects. Many of these studies have only moderate quality evidence, and many improvements were not statistically significant and long lasting.

There is significant mechanistic uncertainty for these findings for several reasons. First, the vast majority of delivered cells die within a few days, and thus are unlikely to contribute to cardiomyocyte mass. Second, the data are limited that bone marrow mononuclear cells and MSC/MPCs develop into endothelial or any other specific cell type as intended, and third, there are very limited data that transferred c-kit+ cells transdifferentiate into cardiomyocytes in humans and remain controversial in preclinical models as well.

Stro-1/Stro-3+ Mesenchymal Precursor Cells for Systolic Heart Failure
In this issue of Circulation Research, Perin et al performed a dose escalating phase II study testing the hypothesis that transcendocardial injection of Stro-1/3+ MPCs would (1) be safe and (2) reduce major adverse cardiac events (cardiac death, revascularization, and nonfatal myocardial infarction–major adverse cardiovascular events [MI-MACE]) in a mixed group of ischemic and nonischemic heart failure patients. The study enrolled 60 people in 3 cohorts of 20 delivering a progressively increasing dose of MPC (25, 75, or 150 million MPCs) via endocardial injection. Within each cohort of 20 patients, 15 were randomized to the intervention and 5 randomized to a mock-injection control group for a total of 45 subjects injected with MPCs and 15 control subjects who were not.

There was no increase in mortality because 5 of 45 MPC-injected subjects died (3 of cardiac causes) versus 4 of 15 control subjects (3 of cardiac causes). There were also no statistical differences in MACE between injected and control subjects (22% versus 33%). Because the cells were allogeneic, immune stimulation was assessed. As assessed by quantification of anti-human leukocyte antigen (HLA) donor-specific antibodies, there were a total of 5 MPC-injected subjects (3 of 15 of the 150 million dose) who had a significant increase in donor-specific antibodies; no subject had any clinical reaction to seroconversion.

Several secondary end points were assessed without evidence of statistical differences including changes in left
ventricular chamber volume and ejection fraction as assessed by serial echocardiography and multigated acquisition scan (MUGA), changes in ischemic burden as assessed by single-photon emission computed tomography, changes in 6-minute walk test, B-type natriuretic peptide (BNP) levels, Minnesota Living Heart Failure questionnaire responses, and New York Heart Association (NYHA) level. For each end point, each treatment group was compared individually and as a pool MPC group versus control, most were assessed serially over time. Some end points (HF-MACE) were compared between treatment groups and individually versus control (HF-MACE). Few secondary end points reached statistical significance, including left ventricular ejection fraction, in the 25 million MPC group at the 3-month time point; however, this difference was not present at the 6- or 12-month time points. In addition, there was a trend toward a small improvement in 6-minute walk test in the 150 million MPC group versus control. The composite end point HF-MACE (cardiac death, heart failure hospitalization, and resuscitated ventricular arrhythmia) was added after the trial began. In comparison with the control group, the 150 million MPC group had increased freedom from HF-MACE, \( P = 0.03 \), by log-rank test.

Based on these results, Perin et al\textsuperscript{17} conclude that transendocardial injection of these proprietary MPCs is (1) safe and (2) reduces heart failure–related MACE at a dose of 150 million MPCs. A concern about this conclusion is that although HF-MACE was compared between each group and against the control with \( t \) tests, no correction for multiple comparisons was used. Multiple comparisons were similarly made among the secondary end points and therefore one should be cautious in using these data as primary justification of efficacy. Moreover, as specifically outlined in the study, the HF-MACE end point was generated in a post hoc manner. In the absence of a type I error, one would expect to see other end points, surrogate markers or logical trends toward improvement in other end points that predict heart failure hospitalizations (the majority of HF-MACE events). More specifically, there were no changes in EF, ventricular volumes, BNP, 6-minute walk test, or NYHA class during final end point analysis (6 months for MUGA and single-photon emission computed tomography, and 12 months for all others). A final concern with the interpretation of the data lies in the study design: the control group did not receive any injection of vehicle, PBS, or non-MSC. Therefore, the reader could conclude that injecting cells into the myocardium stimulated inflammation and thus potentially relevant biological effects. Indeed, the 150 million MPC group received the highest number of injections. However, likely because safety was established, the Food and Drug Administration has approved a phase III study determining the effect of transendocardial injection of 150 million MPCs in heart failure subjects.

### Mechanistic Clues

As the field moves forward with larger studies based on these smaller studies with variable results, determining the mechanism of action in humans becomes that much more important. In that regard, the allosensitization data in Perin et al\textsuperscript{17} are an important consideration. Allosensitization is the generation of antibodies to foreign antigens, primarily MHCI and II proteins. Although the authors downplay the consequences of allosensitization from this therapy, it should not be understated in a patient population who may require cardiac transplantation. The presence and degree of allosensitization directly correlates with long transplant wait times, heart transplant rejection, morbid procedures, and therapies to treat rejection and death.\textsuperscript{18} This is especially problematic in women after childbirth and those who undergo ventricular assist device implantation; female sex and pre–ventricular assist device allosensitization are independently associated with higher postoperative rates and severity of allosensitization.\textsuperscript{19} Nearly 20% of the subjects in the 150 million group had significant allosensitization, similar to other trials of MSCs. In POSEIDON,\textsuperscript{20} a prior study evaluating the safety and effectiveness of allogeneic MSCs in heart failure, 2 of 14 in the allogeneic MSC group exhibited new allosensitization. Ascheim et al\textsuperscript{21} reported HLA sensitization in 2 of 18 subjects after transcendocardial injection of 25 million MSCs.

Although an immunologic response to allogeneic MSC therapy is concerning, it may also provide a potential clue to the mechanisms by which cell therapies may improve left ventricular remodeling.\textsuperscript{22} An intriguing hypothesis is that dying cells provide immune-modulatory signals to the myocardium that alter fibrosis.\textsuperscript{23} The trend toward increased alloimmunization with higher cell counts injected is consistent with the hypothesis that the death of high numbers of allogeneic cell production can stimulate the cardiac immune system in human subjects. Although previously thought to be nonimmunogenic, data support that apoptotic cell products are taken up by antigen presenting cells and activate T-cell subsets.\textsuperscript{24} Thus, allosensitization and immunomodulation could be linked by dead injected cell products (Figure). Future studies that include sensitive tracking of alloimmunization, correlation with total cell 

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**Figure. Injected cells can become apoptotic.** When apoptotic cells are phagocytosed by macrophages (Mac), anti-inflammatory pathways are stimulated. When phagocytosed by dendritic cells (DC), antigens can be presented with major histocompatibility complex (MHC) and stimulate alloimmunization.
injection numbers, and querying serum for novel mechanisms of immune modulation, such as cell-free DNA or exosomes, could provide important insights into these mechanisms.

In a search for the mechanisms driving improved cardiac function after transplantation of bone marrow–derived stem cells, we performed a small hypothesis generating study determining the effect (at the tissue level) of bone marrow–derived fractions injected at the time of left ventricular assist device insertion and analyzed injected tissue harvested at transplant. This pilot study enrolled a small number of subjects, they were prospectively enrolled as their own controls because each subject was injected with saline control and 3 separate bone marrow mononuclear intervention groups in 4 separate regions of ischemic, viable myocardium. In contrast to the results of preclinical trials, we found no increase in endothelial density between areas injected with the saline control in comparison with areas injected with the different bone marrow fractions. However, there were decreased α-smooth muscle actin+ fibroblasts in myocardium injected with either CD34+ stem cells or a CD34-depleted cell preparation when compared with PBS-injected tissue. Supporting the hypothesis that apoptotic cells modulate innate immunity in the heart, in subjects in whom cells were labeled with iron nanoparticles before injection, nanoparticles were found in cardiac macrophages at the time of tissue explant. Accurately characterizing the inflammatory milieu, fate of transplanted cells and metabolic/proteomic profiles from human subjects injected with the cell product will help to identify important cell-independent and cell-specific mechanisms of action.

Although initiated as a therapy to replace cardiac cells, further research with MCS cell therapy has uncovered novel paracrine mechanisms modulating the fibroinflammatory axis in the heart. As large phase III trials of efficacy are initiated, we feel it is imperative to incorporate substudies to determine mechanisms of action in the diseased human heart. We predict that through these studies, the development of lesser immunogenic, more targeted and robust therapies will be appropriately accelerated.

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None.

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