The Editor's Roundtable: Advances in Stem Cell Therapy for Treatment of Cardiovascular Disease

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Introduction

The first clinical trials of stem cell (SC) treatment for cardiac disease in humans, using autologous bone marrow cell transplantation in patients with severe chronic heart failure (HF), were reported in 2002 (using the intracoronary injection route) and in 2003 (using the intramyocardial injection route).[1,2] Since those initial reports, numerous additional clinical SC trials have been conducted in the treatment of cardiac disease, with approximately 30 trials under way in the United States in 2012. In addition to bone marrow–derived hematopoietic SCs, subsequent clinical trials have used mesenchymal SCs, endothelial progenitor cells, skeletal myoblasts, induced pluripotent SCs (and derivatives), and cardiac-resident SCs.[3]

Currently, the 3 main indications for SC treatment in patients with heart disease are chronic ischemic HF, acute myocardial infarction, and idiopathic dilated cardiomyopathy.[4] On the basis of trials over the past decade, "cell therapy may transform the treatment of acute and chronic heart disease, with an anticipated impact rivaling the results of revascularization and reperfusion therapies developed in the last 50 years."[5]

In this Editor's Roundtable, the faculty discusses many issues surrounding this new approach to the treatment of advanced cardiac disease, with particular focus on the use of adipose-derived mesenchymal SCs.

Dr. Friedewald: Let's begin by contrasting *embryonic* SCs with *adult* SCs.

Dr. Hare: The use of embryonic SCs is a very "ethically challenged" arena, especially because major religious beliefs prohibit their use at all. This has placed research with adult SCs at the forefront. Treatment with adult SCs is not opposed on any moral grounds that I am aware of, although the lay population sometimes confuses embryonic with adult SC types. Research with adult SCs has virtually eliminated the need for the use of embryonic SCs in cardiovascular medicine, as we have learned so much about the favorable effects of cardiac repair using cells from bone marrow, from adipose tissue, and from the heart itself. Thus, science has transcended the ethical debates around embryonic SC research.

Dr. Miller: This is a highly controversial field. We have been particularly interested in umbilical cord cells, which also circumvent the controversy surrounding embryonic SCs. Whether umbilical SCs are more robust than adult SCs is unknown.

Dr. Hare: In addition to their being opposed due to religious convictions, research in recent years also has uncovered a significant scientific fact: embryonic SCs form teratomas, which are the in vivo counterparts of teratocarcinomas.[6]

Dr. Willerson: I also share the notion that adult SCs have been surprisingly good, but they often fail when used in older patients. As SCs age, they lose their ability to reproduce themselves. We are trying to develop methods for "resuscitating" them, as there is a beautiful, elegant system of SCs comprised of resident cells in every organ of the body, including the heart. At the time people most need them, with advancing age, they are, however, dysfunctional. I believe that in many persons greater than age 60 years with HF, the underlying pathogenesis of the disease is the inability of myocardial SCs to be functional.

Dr. Friedewald: Do allogenic SCs have a role in cardiology?

Dr. Willerson: Yes, they probably do. Allogenically-transplanted *mesenchymal* SCs are not rejected,[7] except perhaps in a very small number of recipients who may have transient activation of their immune systems, but this may not have clinical significance. Mesenchymal SCs are, in my opinion, 1 of the 2 most effective adult SCs that improve

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blood flow in repair of cardiac disease. The other cell that holds promise is the c-kit cell.

Dr. Friedewald: What are the best sources of mesenchymal SCs?

Dr. Willerson: Both adipose tissue and bone marrow are excellent sources.

Dr. Friedewald: What cardiac conditions respond to SC therapy?

Dr. Willerson: Clinical trials to date have shown that ischemic cardiomyopathy and HF can be treated with SC therapy. Other indications are likely to emerge with further research.

Dr. Friedewald: Let's discuss SC harvest.

Dr. Walpole: A lot of past research has focused on harvesting SCs from bone marrow, but there is a growing body of knowledge about harvesting them from adipose tissue, which offers significant advantages that we will discuss later. Another source for the harvest is the heart itself. This may be particularly important in treating patients with acute myocardial infarction, and quite a bit of recent data has come out in this area, particularly from CADUCEUS (Cardiosphere-Derived Autologous Stem Cells to Reverse Ventricular Dysfunction) trial.[8] CADUCEUS showed that intracoronary injection, 2 to 4 weeks after myocardial infarction, of autologous cardiosphere-derived SCs is a safe procedure and results in "unprecedented" increases in viable myocardium.

Dr. Roberts: What are "cardiospheres"?

Dr. Walpole: Cardiospheres are multiple progenitor cells in spherical aggregates grown from myocardial biopsies and then reintroduced into the patient.[9]

Dr. Hare: Cardiac SCs also can be obtained from right atrial appendage biopsy during cardiac surgery.[10] An exciting future possibility is to mix mesenchymal SCs and cardiac SCs, mutually reinforcing their potential benefits.

Dr. Roberts: How much tissue is obtained from the heart, and where are SCs located in the heart?

Dr. Hare: Cardiac SCs were initially thought to be in specific, niche reservoirs, particularly the right atrial appendage. They also are found in regions close to the atrioventricular node and at the cardiac apex. Other areas of the heart, such as the ventricular septum, which would be easily accessible for biopsy from the right ventricle, are less rich in SCs, but they can be harvested from these sites and propagated in the laboratory, as in CADUCEUS.[8] One definition of SCs is their ability to self-replicate, and all approaches to growing SCs take advantage of cell replication by expanding the number of cells in cell culture. We discovered in the laboratory that mixing heart tissue with bone marrow mesenchymal SCs in cell culture enhances SC proliferation by sixfold. This observation was the origin of our hypothesis that the cells interact with each other and therefore might form an enhanced therapeutic product, which we have proposed for a new clinical trial.

Dr. Friedewald: How does a stem cell transform into a myocardial cell?

Dr. Hare: That goes back to the 2-part definition of a stem cell: (1) self-replication and (2) differentiation. Differentiation goes through a variety of steps, beginning with a stem cell that is not already lineage committed. The first step for a cell to become *lineage committed* occurs when it starts to express transcription factors, signifying that it is committing to a lineage. For cardiac SCs, we know that Nkx2.5 and Gata4 are 2 transcription factors that mark lineage commitment. A lineage-committed cell that is going to mature into a myocyte next goes through a stage in which it is called a *transient-amplifying cell*, which is a premyocardial myocyte with ability for mitosis and division. That cell then matures into a *cardiomyocyte*. Transient-amplifying cells are abundant in adult mammalian hearts, which proves that cardiac regeneration occurs in adults. Thus, SC injection leads not only to differentiation of the injected cells but also triggers endogenous cardiomyocyte replenishment in the heart, suggesting that cardiac regeneration is possible in the adult mammalian heart.

Dr. Friedewald: If SCs reside in the heart, does this imply that in the normal course of cardiac physiology, they are differentiating into cardiomyocytes as replenishment of dead cells? They are in the heart for good reason, correct?

Dr. Miller: Yes, and this is a complete sea change in our thinking. The basic question this addresses is how do hearts live for 60-plus years if there is apoptosis or programmed cell death? The answer is that there must be ongoing hyperplasia and regeneration that sustains the cardiac mass. This concept was proposed early in research attempting to cause the bone marrow or peripheral circulation to stimulate the heart to seed these cells into the heart, blind to the fact that the heart *already* had a resident SC population. This approach is similar to current efforts with allogenic mesenchymal cells, in which we are trying to turn on the native cardiac repair mechanism more effectively. There are, for example, genes like stroma-derived factor 1 (SDF-1) that may be very important in such signaling to initiate endogenous or native repair mechanisms.

Dr. Hare: The big debate has been has been about the rate of endogenous cellular turnover, which some believe to be as low as about 0.5% per year, a rate that declines with age. Others believe the turnover rate of myocytes is much greater, even as much as 100% every 6 or 7 years.

Dr. Friedewald: A complete turnover rate every 7 years has huge implications.

Dr. Hare: Yes, it does. If the turnover is that fast, the question then becomes, Why doesn't the heart repair itself after an acute myocardial infarction? One argument, on a teleological basis, is that endogenous repair is merely for homeostasis, to keep the heart renewed over time as cells are slowly lost. Apoptotic loss, however, is replaced by regenerating myocytes. It may be that what I call "the tsunami of injury" occurring with a myocardial infarction is so overwhelming that it also destroys the cardiac repair mechanism. The great hope is that cell-based therapy can "kick-start" the normal endogenous repair mechanism.

Dr. Willerson: Within a week of birth, most nonhuman and human hearts are incapable of renewing themselves. Jim Martin in our group discovered the pathway by which such inhibition occurs, and it may be possible to manipulate that pathway and regenerate heart cells in vivo. Age is an important factor in SC function, and research must address this factor. For example, we have found that we can take a biopsy, which may contain some mesenchymal SCs, from the hand, put the fibroblasts from the biopsy into culture, and within 2 weeks, those fibroblasts convert into contract-

ing heart muscle cells. Eric Olson at Southwestern Medical School in Dallas has taken certain transcription factors critical to the development of heart muscle cells and treated mice with infarcts in vivo with these transcription factors and converted fibroblasts in the heart to contracting heart muscle cells.[11] In our studies, we have found that some persons greater than age 60 years have dysfunctional SCs—and show no improvement in cardiac function or blood flow when treated with these cells—but *do* respond to allogenic SC therapy.

Dr. Miller: One of the fascinating things about SC research—and this has been verified in clinical biopsies in almost every nonhuman animal model—is that even if you implant *millions* of cells, within 2 weeks, only 1% to 2% of those cells remain viable. Thus, these cells do not immediately create a new syncytium and a new reservoir of functioning myocytes. Rather, it appears that they are primarily "factories" producing the critical factors (genes) to turn on the repair mechanisms of native cells, or a paracrine mechanism, so the main effect is not dependent on transplanted SCs to remain present and viable in the heart for a long period of time. This challenges us to better understand the signaling pathway, especially what turns it on, and what we can do to amplify such signaling.

Dr. Friedewald: Let's address the use of adipose tissue–derived SCs for treating cardiac disease.

Dr. Walpole: First, adipose tissue is easy to obtain by harvesting cells from the anterior panniculus. The procedure is less painful for the patient, and studies have shown that there are hundreds of times more SCs in adipose tissue, per unit of tissue, than are present in bone marrow. As Dr. Willerson said, it appears that as a patient ages, those numbers probably decrease after about age 60 years. Our belief is that adipose tissue is the best location to obtain SCs, particularly mesenchymal cells, and we can use them quicker for treatment because they do not require growth of more cells in culture.

Dr. Friedewald: Take us through the specific steps in the mesenchymal SC therapy, beginning with harvest from adipose tissue.

Dr. Walpole: The procedure begins with a plastic surgeon performing liposuction, in which approximately 200 cm^3 of adipose tissue is aspirated. That tissue is then processed in a particular way that spins down the cells, using enzymes to dissolve the fat. This provides a small plug of SCs that are reconstituted and injected, usually into the myocardium, on the same day.

Dr. Roberts: You inject them into the left ventricular free wall?

Dr. Walpole: That is correct. The catheter is inserted through the femoral artery, just as when performing a routine coronary arteriogram, then across the aortic valve, and down into the left ventricle. Once within the left ventricle, it is directed into a location in the wall where there is good viable muscle with thickness $\geq$8 mm (for safety purposes). Various types of needles have been tried and more are being designed, all with the objective of making injections into viable tissue, which is the optimal reservoir for native cardiac SCs. The cells track down between the muscle cells, possibly in lymphatic channels, for final delivery. It is important not to inject into the scar, because scar tissue tends to be thinner, with a higher risk for perforation and tamponade.

Dr. Roberts: How much do you inject?

Dr. Walpole: There is about 0.2 cm^3 per injection, with about 15 injections.

Dr. Friedewald: What is the mechanism of action of the injected SCs?

Dr. Walpole: The most likely mechanism is via the mesenchymal cells' paracrine effect, which stimulates angiogenesis. There also is some suggestion that they may turn on some of the native cardiac SCs in a way that helps with regeneration as well.

Dr. Miller: Catheter design has been a very important area of development, particularly for HF because, unlike acute myocardial infarction, in which there is so much inflammation that serves as a "homing signal" for SCs to gravitate or mobilize to the area of injury, chronic HF is not associated with that type of signaling. This is the impetus for developing catheters that deliver SCs directly into the myocardial wall. These catheters have electrogram signals that indicate when the catheter tip is located within the myocardium and also create a map so that the operator can define the infarct zone versus the peri-infarction zone. This visual map allows the operator to precisely target the cell delivery. The technology has come a long way in terms of safety and focusing our ability to direct cell delivery with remarkable precision.

Dr. Hare: I agree that SC delivery systems are very important. We have completed 3 trials and are currently doing a fourth study, using a variety of delivery systems. Over the last 10 years at least 5 different catheter systems have been developed, 3 of which have been withdrawn, and 2 are now available, neither of which are approved for clinical use in the United States. Both of these systems are very practical to use. We have used them in Miami in $>$100 patients, and they are highly safe and effective. The procedure itself is no more complex than a coronary angioplasty or an intracoronary stent placement.

Dr. Walpole: The key to this procedure is being very careful and having the right technology to know the exact location of the catheter tip as well as the location of the myocardial scar tissue. These systems now allow you to import data from either magnetic resonance imaging (MRI) scans or cardiac computed tomograms obtained before the procedure. These preprocedural data help define the location and extent of the scar, and biplane systems allow easy location of the catheter tip.

Dr. Friedewald: What difficulties are encountered with catheter placement in patients with idiopathic dilated cardiomyopathy?

Dr. Walpole: The myocardial damage in such patients is more global than local, and often these ventricles are quite large, making it a challenge to reach the chamber wall and to retain good contact.

Dr. Miller: The ventricular wall may be so thin that keeping the catheter *within* the myocardium for the injection is difficult. With current technology, at least 5-mm ventricular wall thickness is needed to avoid perforation and allow safe SC delivery.

Dr. Hare: I urge everybody to think about SC therapy from a biological hypothesis, not just a clinical hypothesis,

because if you start with a biological hypothesis, you will then design a better strategy that matches the right cell with the right patient with the right delivery system. Our understanding of the biology is that recruitment of endogenous cells is very important. Based on that, we reasoned that in the patient with idiopathic dilated cardiomyopathy, we could achieve a therapeutic effect by injecting only in a single territory, even though the pathology is diffuse. This is our hypothesis in a current study, POSEIDON-DCM (The Percutaneous Stem Cell Injection Delivery Effects on Neomyogenesis Pilot Study–Dilated Cardiomyopathy, Nonischemic). The study comprises 36 patients, and results will be available in about 18 months.

In patients with coronary artery disease, we have focused the injections at the border zone of the infarction and viable tissue, and we have delivered some injections into the scar as well. In a 10-injection strategy, 4 or 5 injections are made into the scar and the remainder at the border zone. The decision for that approach was based on the biological observation that postinfarction tissue regeneration occurs in greatest intensity at the interface between the scar and the viable myocardium, and that in addition to a *regenerative* effect, there is also an *antifibrotic* effect. What we observed in this study was a reduction in the amount of scar tissue. I believe that is very important, because this therapy can cause reverse remodeling of the ventricle. This is exciting because if reverse remodeling is achievable, this is the best predictor of improvement in therapeutic outcomes.

Dr. Miller: I agree with Dr. Hare. Measuring scar mass by MRI and then quantitating significant reductions in the mass is important. The number of patients studied so far is small, but trends show we are beginning to understand how the heart can repair and reverse scar tissue, thereby regaining functional myocardium. Remodeling and reducing left ventricular end-diastolic and end-systolic volumes are very important attributes of SCs' ability to reduce scar mass. These end points reflect reverse remodeling of HF with improved ejection fraction and other measures of ventricular function as well as prolonging survival.

Dr. Friedewald: Dr. Roberts, from the pathologist's standpoint, is this consistent with your thinking about post–myocardial infarction healing?

Dr. Roberts: The importance of this procedure depends on the ability of the myocardium to regenerate and how much regeneration is actually taking place. If it is 1%, it is unimportant, but if it is 60%, that is impressive. I have never seen SCs under the microscope when looking at heart tissue, but I must have and simply did not recognize them. Can you look at a histology section and say, "There is a stem cell"?

Dr. Hare: We are highly influenced by what our paradigm is. If the paradigm 20 years ago was that there were no cardiac stem cells, cardiobiologists performed experiments by digesting a heart to come up with cardiomyocytes and other tissue they knew about, and threw away everything else. The "everything else" consisted of cells in the heart that were not cardiac myocytes and were not blood vessels, but what comprised that tissue has been the subject of many debates, such as how to define "myocarditis."

When we inject labeled SCs into the pig heart, we see reservoirs of tiny round cells located in the interstitium between the myocytes. On a hematoxylin and eosin stain, SCs look like lymphocytes or nondescript cells. I believe they are present, but in such low abundance that it has taken the current paradigm shift to look for them. That shift came from Anversa's work[12] and the work of others by identifying c-kit-positive cells in the heart and then removing them and determining what they did. It is clear that there are reservoirs of c-kit cells in the normal and diseased heart.

Dr. Miller: Another way we are looking at this is by gender differences in terms of what is implanted and the presence of the Barr body in SCs. For example, a new trial involves the use of SC therapy in patients with left ventricular assist devices (LVADs), allowing us to examine human tissue. Among the many patients who have received SC therapy, most have survived and were not in critical condition, so we have not yet had an opportunity to examine mechanisms at the molecular level, as well as SC viability itself.

Dr. Hare: Gender mismatching has been crucial to our shift in understanding the whole field. One of the earliest observations was that in gender-mismatched heart transplantation, subsequent heart biopsies showed that cells within the transplanted heart had the sex chromosomes of the host, not of the donor; this was one of the first observations suggesting that there are cells within the body that repopulate a transplanted organ. We have used that technique experimentally in the laboratory by putting male cells into a female host and then later observing male Y-chromosome-bearing myocytes and blood vessels. This is a good translational tool in the human because it avoids the need to do a genetic label of the cells.

Dr. Miller: In the large body of preclinical work, female SCs are much more effective than male SCs. Thus, as we consider using allogeneic donors for SC therapy, it may be wisest to use them from female donors, because female SCs are substantially superior in viability, survivability, reproducibility, and other attributes. Does female SC superiority relate to why women live 10 years longer than men? There is a lot of other interesting biology that may relate to these phenomena.

Dr. Friedewald: What do we know about the mechanism of action of injected SCs, beginning with the *paracrine* effects?

Dr. Walpole: The paracrine effects are primarily SC effects on creating new blood vessel growth, as well as causing changes in the ischemic zone. Currently, cardiac reperfusion is accomplished by epicardial coronary artery stenting and coronary artery bypass grafting (CABG). There are, however, many coronary vessels that are simply too small for us to treat at the epicardial level, so our vision is that SCs will assist with that process via their angiogenetic effect, thereby reperfusing tissue that is still viable. This also may account for some of the improvement in HF patients.

Dr. Friedewald: What evidence do you have that there is actually angiogenesis occurring with SC therapy?

Dr. Hare: We have shown 2 things in our laboratory. First, we can detect Y-chromosome-bearing endothelium and vascular smooth muscle. In addition to structural changes in the arteries, we also showed, using MRI to assess tissue perfusion, that when we injected bone marrow mesenchymal SCs into the border zone of a myocardial infarc-

tion, there was improvement in tissue perfusion as the first observable phenotypic effect. The net result is a powerful phenotypic physiologic effect on improved perfusion and histologic evidence of enhanced vessel formation. There is, in addition, reduced apoptosis. Thus, all of the things that you would like to see that would go along with reverse remodeling are demonstrable in the experimental setting. The next step is to see how this translates into the clinical setting.

Dr. Miller: One of the most important early observations about the paracrine mechanism was that injection of effluent derived from SCs in culture is as effective as actual SC injection. Thus, a fundamental observation is that what is being *produced* by these cells is the driver of the subsequent benefits. For example, one of these products is SDF-1, which has potent SC mobilizing effects as well as anti-inflammatory and antiapoptotic effects. In one study, SDF gene therapy injections directly into the myocardium in the setting of acute myocardial infarction was associated with a 14% improvement in ejection fraction in patients with the greatest injury, which is 5 to 6 times greater than following SC delivery alone.[13] All we are doing is upstream driving of native repair: turning off apoptosis and inflammation. Exactly how this occurs has great implications.

Dr. Friedewald: What are the anti-inflammatory effects of SCs?

Dr. Miller: There are many possible mechanisms, such as suppressing the expression of inflammatory mediators. This is particularly important in the large border zone around an infarct, which is the area in which myocytes are salvaged.

Dr. Friedewald: In what area of the heart does SC-induced angiogenesis occur?

Dr. Miller: I believe SC-induced angiogenesis occurs throughout the myocardium. When looking at capillary density in biopsy specimens from laboratory animals, you find that neoangiogenesis is driven at both microvascular and small epicardial arterial levels.

Dr. Hare: Angiogenesis occurs primarily at the level of small and medium-sized coronary arteries, not in the epicardial coronary arteries. We would argue that a patient receiving SC therapy should first have epicardial revascularization (i.e., coronary angioplasty, intracoronary stenting, CABG) because if you inject SCs into a completely nonperfused segment, the neovascularization that follows may not have a vascular network to incorporate with. Thus, I believe that this biology will lead back into larger clinical questions about the open-artery hypothesis, which has not been substantiated but may need to be reappraised.

Dr. Friedewald: Please explain the open-artery hypothesis.

Dr. Hare: The open-artery hypothesis proposes that even in the presence of a completed myocardial infarction, it is still efficacious to therapeutically open the culprit, occluded vessel. The Occluded Artery Trial (OAT) addressed this hypothesis and was unable to show a clinical benefit for coronary artery revascularization >3 days after myocardial infarction.[14] Something stimulated that hypothesis, however, and I believe that if you bring successful SC therapy into the mix, you may want to couple SC therapy with the open-artery hypothesis. A clinical trial will be needed to compare the effects of SC therapy in patients with and without the open artery. I believe that open artery is very important for optimal SC therapy.

Dr. Walpole: That is consistent with our observation that when patients are differentiated using viability studies such as cardiac MRI or positron emission tomography, patients with significant viability in a region do best when the epicardial coronary arteries are first revascularized.

Dr. Miller: The route of SC delivery is important. For example, atretic or totally occluded coronary arteries are often so scarred and calcified that a catheter cannot enter them, so other approaches are needed. One interesting technique is a retrograde approach through the coronary sinus, which can be totally occluded for up to 20 minutes, allowing time for installation of SCs into the great cardiac vein. Hopefully, this will allow delivery of the cells into ischemic zones that do not have the ability to otherwise be revascularized.

Dr. Friedewald: Following SC injection, when do you see improvement, and how is improvement measured?

Dr. Hare: These are critical questions, and there are no definitive answers, since early data are only now becoming available. So far there are both negative and positive trials. For example, FOCUS-CCTRN (First Bone Marrow Mononuclear Cell United States–Cardiovascular Cell Therapy Research Network), which had a negative outcome, studied bone marrow SC injections in HF patients with reduced ejection fraction and active ischemia.[15] Using MRI tissue tagging, we studied 8 patients with healed myocardial infarction and without active ischemia who responded to SC therapy with reverse remodeling, characterized by reduction in both systolic and diastolic left ventricular dimensions and restoration of function in the injection zone. A new way we are looking at measuring improvement is the "sphericity index," a quantification of how much the heart has shifted in shape from a football-shaped structure to a basketball-shaped structure. Historically, the best end point has been the ejection fraction, but that measure has not served us well, so I believe that a remodeling index is better. The simplest way to measure remodeling is by using chamber dimensions. Regardless what measure is used—sphericity index, ejection fraction, exercise capacity, MVo_2—the most important question is how these translate into clinical benefit. Ultimately we want to see quality of life improvement, such as fewer hospitalizations. This will enable us to correlate the surrogate end point with the clinical outcome, as was done in the past in the development of biventricular pacing.

Dr. Miller: It is important to look at mechanisms. In the SCIPIO (Myocardial Regeneration Using Cardiac Stem Cells) study, the benefit was seen in ejection fraction from 6 to 12 months postprocedure.[10] SCIPIO suggested continued benefit of SC treatment in changing both remodeling as well as improving ejection fraction and functional capacity.

Dr. Friedewald: When do you see improvement in angina pectoris symptoms after SC injection in patients treated for this indication?

Dr. Miller: Chronic refractory angina improves within 6 months after SC injection.

Dr. Friedewald: Are different types of SCs indicated for different conditions (i.e., HF, refractory angina, reversal of postinfarct remodeling)?

Dr. Miller: We cannot answer the question with current data. Different types of SCs and delivery systems may be required for different conditions. A lot of current research is addressing this question. For example, a new study involves injection of CD34 cells in patients with ongoing myocardial ischemia and reduced ejection fraction; the hypothesis in this study is that the CD34 cell is the optimal cell for such patients. For HF patients, I believe that the mesenchymal SC, whether taken from adipose tissue or bone marrow, is the lead candidate.

Dr. Roberts: What do you believe will be the outcomes in SC therapy for patients with angina pectoris and patients with HF?

Dr. Hare: I predict that it will be shown that SC therapy for patients with angina pectoris will show increased exercise capacity, and for patients with HF a better quality of life with improvement in New York Heart Association functional class and fewer hospitalizations. We have built these end points into clinical SC trials.

Dr. Miller: Another question is whether there should be multiple SC administrations, rather than only one. If benefit is derived from a single SC administration, can improved benefit be attained by reamplifying the mechanism? Trials addressing this question also will be carried out.

Dr. Friedewald: What do you believe about the economics of SC therapy for cardiac conditions?

Dr. Miller: Compared to cardiac transplantation, left ventricular assist devices, and other treatments, SC therapy likely can be economically delivered on a broad scale in a lot of hospitals in the United States to large numbers of patients.

Dr. Hare: I completely agree. This therapy could prevent need for defibrillator implantation, for transplant listing, for left ventricular assist device insertion, and also may reduce hospitalizations. I believe it is going to be a highly cost effective new biological therapy. Cost-effectiveness studies will have to be done prospectively, looking at the correct patients, correct SCs, and correct delivery methods.

Dr. Friedewald: How much training is required to become adept in the technique of cardiac SC therapy?

Dr. Hare: Although some training is necessary, I do not believe that training will be comparable to the extent needed to master other common interventional procedures, such as intracoronary stent implantation. The procedure, however, does have risk, because it is an injection directly into the myocardium.

Dr. Walpole: Less manual dexterity is needed than for complex coronary interventions, such as treating junctional coronary lesions and implanting kissing intracoronary stents. Patience, however, is particularly important. I also believe that we must be very careful in patient selection, especially in knowing the exact left ventricular anatomy, which has not been a great concern of interventionalists for many years. For example, a very large left ventricle poses real challenges to good SC injection. The presence of right bundle branch block also is important, because there is risk for damaging the left bundle branch if the catheter is placed high in the left ventricular septum, potentially leading to complete heart block. With practice, first in an animal laboratory, and proper mentoring with patients, however, the experienced interventionalist can learn proper SC injection techniques relatively quickly.

Dr. Miller: Proper training is very important. The University of South Florida has just built a large learning and simulation center, akin to what we are doing with TAVI (transcatheter aortic valve implantation). There are many nuances in use of the SC catheter, much like there are when performing TAVI. Becoming adept is not simply a matter of attending a course, because both dexterity and extensive case experience are necessary before an adequate skill level is attained. I believe that physicians performing this procedure should be certified.

Dr. Walpole: The manual skills are clearly different. The catheters are different. The hub is much larger: bulky compared to a coronary stent catheter. Its mastery primarily involves training your hands and your eyes to work together, just like when learning to implant intracoronary stents.

Dr. Friedewald: Because there are several SC therapy systems, is individual training also needed when switching to a new system?

Dr. Walpole: Yes. Although the systems are similar, there are significant differences that require individual training. For example, maneuvers directing the tip of the catheter are different with each system, different safety features must be learned, and the curve on the catheter determines the ease in accessing some areas. In addition, even when working with one system, I believe the day will come when one type of catheter is required for one size ventricle and a different catheter for another size. When you perform procedures on animals, you may get a false sense of security, because their ventricles are small. Currently, however, our catheter size options are limited.

Dr. Friedewald: What patient is the "perfect" candidate for SC treatment for HF?

Dr. Miller: This is an important but complex question. Age is a significant issue, because HF prevalence greatly escalates after age 65 years. Thus, it is imperative that cost-effective strategies are developed for this population. Another factor is HF stage. Do we treat only stage IV, or do we prefer to treat stage II to III in order to prevent subsequent hospitalizations and, hopefully, improve the natural history of the disease? I believe that any patient who has become unresponsive or decreasingly responsive to good medical therapy is a candidate. We know that hospitalization for HF while on medical therapy is the most important prognostic indicator, and is a good trigger for when we might want to intervene with SC therapy. Preventing readmission for HF is probably where we have the biggest potential cost and personal benefit.

Dr. Friedewald: Is a 20-year-old patient with fulminate myocarditis and HF a candidate for SC therapy?

Dr. Miller: When you look at recovery rates in patients with HF who receive mechanical devices and drug therapy, the best results are in the youngest patients. This is probably due to better native SC function compared to older persons. They have much more robust repair mechanisms. Thus, I believe that the benefit in SC treatment of older patients, in whom the native repair mechanism is not nearly as effec-

tive, may be where we make the biggest impact with SC therapy.

Dr. Friedewald: What is the efficacy of SC therapy in patients with HF and preserved systolic function?

Dr. Miller: To date, SC treatment has not been studied in HF patients with preserved systolic function. This is an important question, however, because this group represents about half of all patients with HF. Although we do not understand all of the mechanisms of this form of HF, mesenchymal SCs are potentially efficacious by possibly enhancing endothelial and/or myocardial nitric oxide production.

Dr. Hare: We have unpublished data in a subpopulation of postinfarction HF patients with significant diastolic dysfunction and a very steep pressure-volume curve. We found that with a certain SC mixture treatment in these patients there was complete normalization of the diastolic pressure-volume curve. This finding was unanticipated, as it occurred while we were calculating pressure-volume loops to study ventricular systolic function and energetics. Thus, it is possible that SC therapy could improve left ventricular diastolic compliance. As we learn more, we will be able to better tailor the right cell to the right patient with the right delivery method, and this may include patients with diastolic HF. There are many specific infiltrative diseases, such as amyloidosis, that have very poor treatment options at this point, and I wonder whether future SC therapy may benefit these patients as well.

Dr. Roberts: What about sarcoidosis?

Dr. Hare: Sarcoidosis also may be amenable to SC therapy, because it is an inflammatory disease, although we do not know its cause. SCs, particularly mesenchymal SCs from adipose tissue and bone marrow, are powerful modulators of inflammation. Their anti-inflammatory property is important for their use as an allogeneic graft. My colleague Camillo Ricordi showed that co-infusion of mesenchymal SCs with kidney transplantation enhanced the graft acceptance in the donor recipients, a beautiful and direct demonstration of the immunomodulatory features of the cells, by enhancing graft tolerance.[16] Thus, it is very attractive to speculate that the immunomodulatory effects of SCs may be clinically important, such as treating sarcoidosis.

Dr. Friedewald: How many patients in the United States with HF would be candidates for SC therapy if the treatment were approved today?

Dr. Miller: There are currently 3.5 million people with any form of HF in the United States today, and as many as 350,000 have advanced HF that would fit our current criteria for SC therapy. This number will greatly increase as we learn more about specific SC therapy according to patient age, gender, etiology, and stage of HF.

Dr. Hare: You could argue that anybody with some type of myocardial injury may benefit, because you might prevent remodeling before it occurs, even patients with relatively small myocardial infarctions. As always in cardiovascular medicine, we initially use a new treatment modality in the sickest patients, especially patients with poor options or no options. With time and better definition of treatment, such modalities are then applied to patients who are less sick. This is analogous to the use of statins (3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors), which were initially prescribed only for patients with marked hypercholesterolemia, and are now widely used in primary prevention for persons with much lower cholesterol levels.

Dr. Friedewald: I understand that bathing SCs in a statin solution prior to injection enhances their therapeutic effect.

Dr. Hare: Yes, that is correct. John Canty's group at Buffalo showed that statins enhance SC cell effects, particularly cardiac SCs.[17] This observation points out the need to further study interactions between SC therapy and conventional treatment strategies.

Dr. Friedewald: Do you routinely prescribe a statin before the procedure?

Dr. Hare: No, that is not part of current protocols. We have, however, criteria for best conventional therapy. For example, HF patients need to be on an optimal regimen including a renin-angiotensin-aldosterone system antagonist, a β blocker, and, if indicated, a statin. Thus, our approach is to superimpose cell therapy on the best medical therapy as well as revascularization: intracoronary stenting and CABG.

Dr. Walpole: These patients also need a lot of education on risk factor modification as part of an ongoing effort to slow progression of coronary disease. With treatment of patients who are less sick and less symptomatic, we will have to develop ways to deliver the cells at lower risk, as intramyocardial injections carry potential significant risk. For example, smaller catheters would permit a radial artery approach and, as mentioned earlier, delivery into the coronary sinus via a femoral vein approach would also probably have less risk.

Dr. Miller: The scope of cardiovascular diseases treatable with SC therapy also is 1 in 10 million; at age 40 years, it is 1 in 40 million; at age 60 years, it has fallen to 1 in 60 million. Regardless of the indices used to measure SC functions, there is no question that aging is accompanied by a decline in the body's ability to compensate for normal cell attrition or to handle any significant acute insult, at least in bone marrow as the source. There is some encouraging evidence, however, that an age-related decline in the number of SCs does not occur in adipose tissue. If this is true, adipose tissue may be a better SC source in patients greater than age 65 years. This possibility may heavily influence our efforts to develop allogenic sources of SCs.

Dr. Friedewald: Is failure of the body's ability to replace myocardial cells the etiology of HF in many older patients, even patients with no prior history of ischemic or valvular heart disease?

Dr. Miller: It is plausible.

Dr. Hare: The paradigm has shifted from an assumption that we die with the same cardiomyocytes that we are born with. Now we recognize a more dynamic, homeostatic mechanism for *all* of our organs, where there is slow cell loss coupled with constant replenishment. This process of cell replacement is true even for brain cells.

Dr. Friedewald: Earlier studies using skeletal muscle SCs were complicated by ventricular arrhythmias.[18] Is this a concern with other types of SC therapy?

Dr. Miller: I worked with other early investigators using skeletal myoblasts. We reasoned that because the heart is a skeletal muscle, use of skeletal myeloblasts was logical. When injected into the heart, however, skeletal myoblasts

do not differentiate into true functioning cardiomyocytes, and they are unable to electrically connect to the true myocardium. They also are quite proarrhythmic, an effect that has not been observed with other types of SCs.

Dr. Friedewald: Why are skeletal myeloblasts proarrhythmic?

Dr. Miller: Following implantation into the myocardium, skeletal muscle cells do not form gap junctions, which are necessary for myocardial electrical synchrony. The implanted tissue becomes an irritant, creating an electrical loop that leads to ventricular tachycardia. This phenomenon occurs within 2 weeks after implantation. In contrast, mesenchymal SCs have been used to *treat* ventricular tachycardia in nonhuman animal models. This could be due to their anti-inflammatory effect, and raises the possibility that SC therapy could be a future treatment of cardiac arrhythmias.

Dr. Friedewald: Have you seen any improvement in cardiac dysrhythmias in human trials with SC therapy?

Dr. Hare: Yes. In a study of patients with acute myocardial infarction treated with intravenous mesenchymal SCs, there was about 90% reduction in arrhythmic events identified by ambulatory monitoring for 12 months.[19] This was one of the early observations that mesenchymal SCs are antiarrhythmic, contrary to concerns we discussed earlier that they might be proarrhythmic. Mesenchymal SCs are a rich source of connexin-43, which is the protein involved in gap junction formation, so they form gap junctions with host myocardium, in contrast with proarrhythmic skeletal muscle cells.

Dr. Friedewald: What is next, in both the immediate and long-term future of SC therapy for cardiac disease?

Dr. Hare: There is a very rapid proliferation of enthusiasm for this therapy among both the medical community and among patients. The medical community has a major responsibility to aggressively perform the appropriate studies that could lead to clinical approval of this therapy. Some forms of SC therapy have been approved in Europe. I predict that we will have approval in the United States for SC treatments sooner than most experts believe, perhaps within the next 3 to 5 years. One of the most important initiatives is the Cardiovascular Cell Therapy Research Network (CCTRN), funded by the National Institutes of Health. The CCTRN has devoted substantial resources to a multicenter network that Dr. Willerson, Dr. Miller, and I are honored to participate in. In this consortium, SC research trials can be conducted very quickly.

Dr. Miller: At last count, there were 27 major ongoing SC clinical trials in the United States in several different areas of cardiovascular disease. From this will emerge a substantial body of evidence that I believe will accelerate the regulatory approval process.

Dr. Roberts: If you can prevent the use of left ventricular assist devices, heart transplantation, and similar hugely expensive treatments, SC therapy has a great future.

Dr. Hare: You can envision a coupling of SC therapy with other conventional, often expensive modalities used to treat HF. Envision this: all the patients who are evaluated for heart transplants could be submitted, with their consent, to SC therapy first. This may prolong their need for transplants or may reverse the need. The same approach could be used in patients who are candidates for LVADs and intracardiac defibrillators, which are now indicated based on ejection fraction. Perhaps you first treat patients with cell therapy; if they have a good response as measured by ejection fraction, they then come out of the conventional indication for defibrillation, but within the coupled strategy. At a later stage, should their ejection fraction deteriorate, they can then get defibrillators. This general approach could be very cost saving to the health care system.

Dr. Walpole: This approach would be particularly applicable to acute myocardial infarction patients, in whom there may be some improvement in ejection fraction anyway. If you can improve that even further, the incidence of ventricular arrhythmias and subsequent need for intracardiac defibrillators may be decreased, thereby reducing the need for a lot of very expensive technology.

Dr. Miller: Currently, cardiovascular disease costs the United States an estimated $280 billion per year and is projected to triple in the next 20 years due to increasing prevalence of cardiovascular disease as the population ages. These data are a mandate to develop new therapies to change that trend, and SC therapy could become one of the best new therapeutic options because it carries the potential to benefit patients with almost all forms of cardiovascular disease.

Dr. Friedewald: What are the safety issues with SC therapy, other than with the delivery technique itself?

Dr. Hare: There are some important things to be considered, which are in part theoretical but also quite concerning. For example, there is an interface between regenerative medicine and cancer. There is a stem cell hypothesis of cancer that is based upon the notion that cancer originates in SCs that have gone awry. In experimental animals, SCs can lead to ectopic tissue formation—differentiating into an unwanted lineage—and/or stimulating tumor growth. As SC therapy comes into greater use, with more clinical trials, it will be very important to screen patients for cancer. Currently, we do assays for prostate-specific antigens and take careful histories for cancer. Patients with cancer during the previous 5 years are excluded from cell trials on the premise that SC therapy could activate an occult tumor or make a recurrent tumor more aggressive. There have been studies in which breast cancer cells are co-cultured with mesenchymal SCs, either from adipose tissue or bone marrow, and there is an enhanced proliferation rate of cancer. In theory, a patient with prior cancer could be exposed to an enhanced risk for tumor recurrence. We need data to address that issue. I want to emphasize, however, that this concern is only theoretical and has not been reported. Nonetheless, cancer risk must be carefully studied, including long-term registry follow-up.

Dr. Friedewald: Once a patient has been admitted to the hospital for HF, however, the 5-year mortality is about 50%, which is a worse prognosis than breast or colon cancer.[20] What is the fate of SCs injected into the heart?

Dr. Hare: In experimental situations in which SCs are tagged, only about 20% of the injected cells remain in the heart. Cell retention may be influenced by the delivery method and catheter design.

Dr. Roberts: What is the fate of the SCs that are not retained in the heart?

Dr. Hare: Most of them are trapped in the lung and then slowly washed out. In animal studies, they cannot be found anywhere in whole-body autopsies. This does not necessarily imply, however, that they do not continue to have functional benefits after washing out of the heart. Even with a short stay in the heart, SCs can interface with host mechanisms and trigger regenerative responses. Our program is looking not only at cardiac function in patients who have received SC injections, but whole-body function as well. For example, an important observation from the Osiris study, which addressed our concern that cell therapy could impair lung function, found the opposite: the FEV_1 (forced expiratory volume in 1 second) was enhanced in patients after SC injection.[19] That finding led to more interest in treating patients with chronic obstructive pulmonary disease with mesenchymal SCs. Investigators also are considering cell studies for idiopathic pulmonary fibrosis, which has the perfect biology to be responsive to SC therapy.

Dr. Miller: SC therapy needs to be studied on a very thoughtful basis so that we learn the important lessons that will lead to its effective commercialization.

Dr. Walpole: The biological strategy and the underlying mechanism by which SCs have an effect are important, as we have learned in other areas of cardiac disease. When we developed drug-eluting intracoronary stents, for example, we learned that the drug's action occurs in a certain, finite period of time, and does not have to remain permanently for it to do its intended job. The same may be true for SC therapy.

Dr. Friedewald: Closing comments?

Dr. Hare: This is a very exciting time in medicine, cardiovascular medicine in particular, because we have hit a treatment logjam in our specialty. Although we have seen a nice reduction in cardiovascular mortality over the last several decades, until now, we have failed to develop the means to regenerate tissue. I believe, however, that we are starting to peel back the layers of that onion. For the first time we can conceptualize strategies for tissue regeneration in the myocardium, blood vessels, and other body tissue. These strategies can be highly cost effective. I emphasize that we are at the end of the beginning, not at the beginning of the end of SC research. We have a lot of work ahead of us, but fortunately, there are many, many investigators and practitioners around the United States and the world who are very excited about cell therapy, and we will see some very important answers in the next 3 to 5 years.

Dr. Miller: I agree. Most drug therapy for cardiovascular disease has been based on finding circulating peptides that appear to be targets associated with increasing levels in the blood, with progressive severity of diseases such as HF. Yet there are many drugs that made good sense and worked in the preclinical setting but have not been effective in the clinical setting, which reinforces the need for new approaches to cardiovascular treatments. With SC therapy, we are working with the body's native repair mechanisms. Our challenge is to understand how to harness those mechanisms and how to turn them on effectively, which is fundamental biology in human science. I believe that SC therapy makes more sense than almost anything we have done before, and we must learn how to use it most effectively.

Dr. Walpole: We all recognize that the rising cost of health care in the United States is unsustainable. The number one DRG (diagnosis-related group) in the United States is HF and its complications. Thus, if we can devise ways to economically reduce the incidence of HF, reduce the incidence of ventricular arrhythmias associated with HF, and improve the quality of life of patients with HF, then we will have accomplished a great thing. I believe we will do that with SC therapy.

Dr. Roberts: I have been a cynic of SC therapy in the past, but this discussion virtually reversed that thinking today.

Dr. Friedewald: Thank you.

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