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EDITORIAL COMMENT

Cell Therapy Needs Rigorous Translational Studies in Large Animal Models*

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Cell therapy, arguably the most exciting field of research in contemporary cardiovascular medicine, is mired in controversy and uncertainty (1,2). One of the major obstacles to progress is the paucity of rigorous translational studies, particularly studies in large animals (1). Understandably, the initial evaluation of new cell therapies is usually conducted in rodents, but what should come next? Assessing every new type of cells in humans would not be safe, practical, or cost-effective. Before moving to clinical trials, promising cell types must be evaluated in clinically relevant, large animal models.

At present, however, the majority of research dollars allocated to preclinical trials of cardiac regeneration subsidize studies in rodents; only a small minority of available grants supports translational work in large animal models that are more relevant to humans. As a result, most of the preclinical knowledge is predicated on data obtained in rodents, particularly mice. Murine models are relatively cheap and quick and lend themselves to genetic manipulations, but are they relevant to humans? Are the effects of stem/progenitor cells the same in mice and humans? The answers to these questions are unknown. Evidence that murine models are similar to the human situation is scarce; if anything, there is considerable evidence to the contrary. For example, mice and humans, separated by 65 to 100 million years of evolution, differ significantly with respect to innate and adaptive immunity (3), sarcomeric proteins (e.g., predominantly MYH7 myosin isoform in humans vs. MYH6 isoform in mice), and ion channels (e.g., mice lack IKr), not to mention the gargantuan influence of genetic backgrounds in different murine strains. Human hearts do not beat 500 to 700 times/min, and repair of human myocardial infarctions requires replacement of several grams of dead tissue, not a few milligrams. Given these enormous differences, the willingness of some investigators (as well as the lay public and media) to readily extrapolate murine data to humans is, indeed, surprising. For example, mouse studies have been glibly used as a basis for recommending that clinical trials of cell therapy be stopped, which would be irrational. Of course, murine models can be useful as screening tools and to interrogate molecular mechanisms, but one must always keep in mind that the actions of stem/progenitor cells may be completely different in humans.

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Given the obvious limitations of murine models, it is crucial for the progress of the field that cell therapy be studied in large animal models that are closer to the clinical setting. In this issue of the *Journal*, Karantalis et al. (4) report that the combination of mesenchymal stem cells (MSCs) and c-kit$^+$ cardiac progenitor cells (CPCs) was superior to MSCs alone in improving global left ventricular (LV) performance in a porcine model of chronic ischemic cardiomyopathy; both treatments, however, achieved a similar improvement in wall motion in the infarct zone and a similar reduction in scar size. This group had previously found that human CPCs and human MSCs have additive effects in repairing infarcted myocardium in pigs (5). Compared with that earlier study, the present research is a significant advance because of several aspects that increase the clinical relevance of the observations. First, the authors used autologous

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porcine cells as opposed to xenogeneic human cells, thereby obviating the need for immunosuppression, which could alter the response of the host to the transplanted cells and has no clinical correlate. Second, the cells were delivered transendocardially with the NOGA system for electroanatomic mapping (Biosense Webster, Inc., Diamond Bar, California) as opposed to the transepicardial route (6), which is not feasible or practical in most patients. Third, the cells were transplanted 3 months after myocardial infarction (MI) as opposed to 2 weeks after MI; that is, cell therapy was performed in a setting that simulates chronic ischemic cardiomyopathy rather than acute or subacute MI. An important strength of this study is that it was conducted in a well-established porcine model (7), with the use of state-of-the-art methods and a demanding protocol lasting 6 months. Taken together, the findings of Karantalis et al. (4) provide further evidence that combining MSCs with CPCs results in greater efficacy than using MSCs alone for the treatment of post-MI LV remodeling and dysfunction, even when the healing process is complete and the disease has entered its chronic phase.

The findings of Karantalis et al. (4) have clear therapeutic implications. Phase I clinical studies have suggested that both CPCs (8) and MSCs (9) alleviate LV dysfunction, reduce scar size, or both; if the results of the present study are applicable to humans, the effectiveness of cell therapy in patients with chronic ischemic heart failure would be enhanced. In conjunction with a previous study by this group (5), the observations of Karantalis et al. reinforce the rationale for the soon-to-be-initiated CONCERT-HF (Combination of Mesenchymal and C-kit+ Cardiac Stem Cells as Regenerative Therapy for Heart Failure [NCT02501811]), a Phase II trial by the Cardiovascular Cell Therapy Research Network that will compare the safety, feasibility, and efficacy of MSCs alone, CPCs alone, and their combination in patients with ischemic cardiomyopathy.

Few studies have combined 2 cell types at the experimental level (5,10–18) and none at the clinical level (**Table 1**). In general, these studies found that combination therapy was superior to single-cell therapy. As indicated earlier, Williams et al. (5) administered human CPCs, human MSCs, or their combination in a swine model of subacute MI. Left ventricular ejection fraction was restored to baseline with both single and combination therapy. However, the reduction in infarct size was double with combination therapy compared with single-cell therapy; in addition, the engraftment of transplanted cells was 7-fold greater with the combination therapy than with either cell type alone. Using human saphenous vein-derived pericyte progenitors (a subtype of MSCs) and CPCs in a murine model of acute MI, Avolio et al. (10) showed that both single-cell and dual-cell therapy led to an improvement in left ventricular ejection fraction and a reduction in interstitial fibrosis; however, only the combination therapy resulted in a reduction in infarct size and an increase in arteriogenesis compared with vehicle. Similarly, Winter et al. (11) reported that the combination of epicardial-derived cells and cardiomyocyte progenitor cells was superior to single-cell therapy. In yet another study, Latham et al. (12) demonstrated in a murine model of acute MI that the combination of human circulatory angiogenic cells and cardiac stem cells resulted in greater improvement in left ventricular ejection fraction, a reduction in infarct size, and an improvement in capillary density compared with single-cell therapy. The positive interaction between 2 cell types reported in these studies (5,10–18) was presumably the result of paracrine mechanisms. Taken together, the present report (1) and these previous studies (5,10–18) support the concept that combinatorial therapy is likely to be superior to single-cell therapy, and it should therefore be one of the main avenues for future research.

The work of Karantalis et al. (4) is also important because it epitomizes rigorous translational research. Translational studies in large animal models (usually pigs) are rare because they are expensive, complex, time-consuming, technically demanding, slow, and usually not suitable for mechanistic investigations; nevertheless, because they are conducted in settings closer to the human situation than those found in rodent models, these studies are essential to justify the risks and costs of clinical trials. Unfortunately, the added value of the clinical relevance of large animal models is often not appreciated by reviewers of manuscripts and grant applications, who assign low scores on the basis of lack of mechanistic insights and insufficient conceptual novelty. For cell therapy to be translated into clinical therapies, it is critical that this misperception be corrected.

It is also critical that access to large animal models be available to the entire scientific community because very few investigators have the expertise necessary to use such models successfully. While the number of molecular and cellular biologists working on stem/progenitor cells continues to increase, the number of integrative physiologists continues to decrease inexorably. These considerations provide a cogent rationale for the National Institutes of Health to establish a national consortium of core laboratories

TABLE 1 Summary of Experimental Studies of Combination Cell Therapy

First Author (Ref. #)	Host	Type of Heart Disease	Study Groups	No. of Animals per Group	Time of Cell Therapy	Dose and Route of Administration	Follow-up Period After Cell Therapy	Final Assessment Modality	Outcome
Williams et al. (5)	Yorkshire pigs	Subacute MI	hMSCs; hCPCs; both hMSCs and hCPCs; placebo	5	2 weeks after MI	Intramyocardial via minithoracotomy; hMSCs 2×10^8; hCPCs 1×10^6; dual therapy included both the above doses	4 and 6 weeks	MRI Micromanometer Conductance Catheterization	EF ↑ in all cell groups Infarct size ↓ in all cell groups (dual therapy > single therapy) Diastolic function ↑ in dual therapy > single therapy Stem cell engraftment↑ in dual therapy >> single therapy Contractility (preload recruitable stroke work and dP/dt_{max}) ↑ in dual therapy > single therapy
Avolio et al. (10)	SCID-beige mice	Acute MI	hSVPs; hCPCs; both hSVPs and hCPCs; vehicle; sham	5–7	At the time of MI	Intramyocardial via thoracotomy; hSVPs 3×10^5; hCPCs 3×10^5; dual therapy included both the above doses	2 and 6 weeks	Echocardiography Intraventricular pressure measurement	EF ↑, interstitial fibrosis ↓ in all cell groups LV remodeling ↓ in dual therapy and hCPC group Stem cell engraftment in dual therapy ↔ Infarct size ↓ and arteriogenesis ↑ in dual therapy > single therapy
Winter et al. (11)	Diabetic/ SCID mice	Acute MI	CMPCs; EPDCs; both CMPCs and EPDCs; vehicle; sham	13–20 (3 in sham)	At the time of MI	Intramyocardial via thoracotomy; CMPCs 4×10^5; EPDCs 4×10^5; dual therapy included a total of 4×10^5 CMPCs and EPDCs	6 weeks	MRI	EF ↑ in all cell groups (dual therapy > single therapy) EDV and ESV in dual therapy comparable to sham
Latham et al. (12)	NOD-SCID mice	Acute MI	hCACs; hCSCs; both hCACs and hCSCs; NHDFs; vehicle	9–13	1 week after MI	Intramyocardial via echocardiography guidance; hCACs 1×10^5; hCSCs 1×10^5; hCACs and hCSCs in dual therapy 0.5×10^5 each	2, 3, and 15 weeks	Echocardiography	EF ↑ in all cell groups except NHDFs (dual therapy > single therapy) Infarct size ↓ and capillary density ↑ in dual therapy > single therapy Stem cell engraftment in dual therapy ↔
Li et al. (13)	Japanese white rabbit	Ischemic cardiomyopathy	hUC-MSCs; both hUC-MSCs and hUCB-$CD34^+$; vehicle	8	4 weeks after MI	Intramyocardial via thoracotomy; 5×10^6 hUC-MSCs; dual therapy included 5×10^6 hUC-MSCs and 5×10^5/kg $CD34^+$	4 weeks	Echocardiography	LV fractional shortening ↑ dual therapy > single therapy > vehicle Capillary density ↑ dual therapy > single therapy
Ott et al. (14)	Fisher rats	Acute MI	SM; BMMNCs cells; both SM and BMMNCs; vehicle	10–14	1 week after MI	Intramyocardial via thoracotomy; SM 1×10^7; BMMNCs 1×10^7; SM and BMMNCs 5×10^6 each	8 weeks	Echocardiography	EF ↑ in SM and dual therapy groups EDV ↓ in dual therapy but not single therapy compared with baseline Neovascularization ↑ in SM and dual therapy groups > BMMNCs

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TABLE 1 Continued

First Author (Ref. #)	Host	Type of Heart Disease	Study Groups	No. of Animals per Group	Time of Cell Therapy	Dose and Route of Administration	Follow-up Period After Cell Therapy	Final Assessment Modality	Outcome
Memon et al. (15)	Beagle dogs	Acute MI	SM; BMMNCs; both SM and BMMNCs; vehicle	4	At the time of MI	Intramyocardial; SM 1×10^8; BMMNCs 3×10^6; dual therapy included both the above doses	4 weeks	Echocardiography	EF ↑ in dual therapy > single therapy Neovascularization ↑ dual therapy > single therapy
Bonaros et al. (16)	Nude rats	Ischemic cardiomyopathy	SM; hAC133$^+$ cells; both SM and hAC133$^+$; vehicle	5	4 weeks after MI	Intramyocardial via thoracotomy; SM 1×10^6; hAC133$^+$ 1×10^6; SM and hAC133$^+$ 5×10^5 each	4 weeks	Echocardiography	EF ↑ in all cell groups; dual therapy > single therapy EDV ↓ in dual therapy > single therapy Infarct size ↓ and capillary density ↑ in dual therapy > single therapy
Suuronen et al. (17)	Sprague-Dawley rats	Subacute MI	MSCs; EPCs; both MSCs and EPCs; vehicle	6-8	3 weeks after MI	Intramyocardial via thoracotomy; MSCs 1×10^6; EPCs 1×10^6; MSCs and EPCs 5×10^5 each	4 weeks	Echocardiography	EF ↑ in EPC group > MSC and dual therapy groups Arteriogenesis ↑ in EPC group > MSC and dual therapy groups Infarct size in all cell groups and vehicle ↔
Bonaros et al. (18)	Nude rats	Ischemic cardiomyopathy	SM; hAC133$^+$ cells; both SM and hAC133$^+$; vehicle	10–12	4 weeks after MI	Intramyocardial via thoracotomy; SM 1×10^6; hAC133$^+$ 1×10^6; SM and hAC133$^+$ 5×10^5 each	NA	NA	Stem cell engraftment ↑ in dual therapy > single therapy Angiogenesis ↑ in dual therapy > single therapy

↑ = increased; ↓ = decreased; ↔ = no change; BMMNC = bone marrow mononuclear cell; CMPC = cardiomyocyte progenitor cell; dP/dt max = the rate of left ventricular pressure rise in early systole; EF = ejection fraction; EDV = end-diastolic volume; EPC = endothelial progenitor cell; EPDC = epicardium-derived cells; ESV = end-systolic volume; hCAC = human circulatory angiogenic cell; hCPC = human cardiac stem cell; hCSC = human cardiac stem cell; hMSC = human mesenchymal stem cells; hUC-MSC = human umbilical cord-derived mesenchymal stem cells; hUCB-CD34$^+$ = human umbilical cord blood-derived CD34$^+$ cells; hSVP = human saphenous vein-derived pericyte progenitor; LV = left ventricle; MI = myocardial infarction; MRI = magnetic resonance imaging; MSC = mesenchymal stem cell; NA = not applicable; NHDF = normal human dermal fibroblasts; NOD = nonobese diabetic; SCID = severe combined immunodeficiency; SM = skeletal myoblast.

that have expertise with large animal models and make these complex models available to the scientific community. With such an infrastructure in place, all investigators would have the opportunity to conduct studies of stem/progenitor cells in relevant and rigorous preclinical models that would otherwise be impossible for them to use. Such an infrastructure would be analogous to the Consortium for Preclinical Assessment of Cardioprotective Therapies, which was developed to study reductions in infarct size (19).

A massive disproportion currently exists between rodent studies and large animal studies. There is a need to increase the number of rigorous and relevant preclinical studies, such as that by Karantalis et al. (4). Until a publicly available consortium is developed to rigorously evaluate stem/progenitor cells in clinically relevant, large animal models, the progress of cell therapy will be hindered, and translation into human therapies will be difficult.

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