EDITORIAL COMMENT

Of Mice and Men
The Best Laid Scheme?*

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More than 5.8 million U.S. adults have heart failure, including an estimated 250,000 to 500,000 with end-stage heart failure refractory to medical management (1). More than 2,400 durable, FDA-approved mechanical circulatory support devices are implanted annually in the United States (2). The rising rate of device implantations reflects the improved survival and quality-of-life benefits that devices offer, although the 2-year survival rates have plateaued at 70% (2). The most recent INTERMACS (Interagency Registry for Mechanically Assisted Circulatory Support) report (2) found that 42% of durable devices are implanted as destination therapy and only 1% of patients with devices implanted as a bridge-to-heart transplantation experienced recovery at 1 year. Preclinical investigations that demonstrate the capability of cell therapy to improve myocardial perfusion and function in both post-myocardial infarction and heart failure models have stimulated optimism regarding the use of cell therapy for patients with advanced heart failure (3–5). In particular, there has been enthusiasm for the use of stem or progenitor cells in patients undergoing left ventricular assist device (LVAD) implantation. This offers the dual opportunity to examine cardiac tissue in patients undergoing heart transplantation to obtain insights into the mechanism of benefit, as well as the potential to improve myocardial recovery (6). Despite evidence from preclinical models and early clinical trials, considerable controversy remains regarding the magnitude of benefit and the mechanisms of effect with cell therapy (7–9).

The first successful report of combined cell therapy and LVAD implantation was in this Journal in 2003, when Pagani et al. (10) treated 5 ischemic cardiomyopathy patients undergoing LVAD implantation with autologous skeletal myoblasts and reported that 3 of 4 patients undergoing cardiac transplantation had evidence of engraftment, including 1 with evidence for small vessel formation. In the ensuing 12 years, ≤30 additional patients with ischemic cardiomyopathy undergoing LVAD implantation in combination with cell therapy have been reported in the literature without further histopathological assessment of neovasculogenesis (6).

In this issue of the Journal, Stempień-Otero et al. (11) report the histopathological results following injection of autologous CD34+, CD34−, bone marrow mononuclear cells, and vehicle into 4 quadrants of a 1-cm diameter area of myocardium immediately before LVAD implantation in 6 patients with ischemic cardiomyopathy (12). Only 7 of 26 patients scheduled for LVAD as a bridge to transplantation were recruited, 1 was too unstable for injection, and another had insufficient CD34+ cells for injection. The period for recruitment of the trial is not reported. Compared with 13 “control” patients who underwent LVAD implantation without injections, the 6 patients receiving cell injections had similar blood product utilization and ventricular arrhythmias within the first postoperative week without a death or reoperation in either group, supporting the feasibility and safety of this approach.

In contrast to the pro-angiogenic, anti-fibrotic, and anti-inflammatory effects reported in preclinical models with CD34− cells, the authors report reduction

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in endothelial cell density in the 5 patients with regions injected with CD34+ cells relative to vehicle and no significant differences in fibrosis or inflammation based on histopathological evaluation at the time of transplantation 42 to 374 days later. They do find a reduction in activated myofibroblasts in both the CD34+ and CD34− segments. These results conflict with previous preclinical studies that describe enhanced neovasculogenesis in animal models of ischemic heart disease. For example, in a rat model of acute myocardial infarction, intramyocardial injection with 5 × 10^5 cells/kg of CD34+ cells resulted in increased capillary density, reduced fibrosis, and preserved cardiac function with higher fractional shortening and regional wall motion scores (6). The extensive preclinical data supporting an improvement in myocardial perfusion with CD34+ cells are supported by clinical trial results, which are consistent with an improvement in myocardial perfusion (7,8). In particular, a double-blind trial using intramyocardial injections of CD34+ cells in 167 patients with refractory angina resulted in a significant improvement in angina and exercise tolerance (12).

So, how do we explain this discrepancy between “mice and men”? Preclinical models are designed to maximize homogeneity with same sex, genetic makeup, age, and absence of underlying coronary atherosclerosis, cardiovascular risk factors, and medications, which confound clinical trial results. Is this a case in which the preclinical models are not pertinent and/or misleading, or are there limitations with the human model?

Failure to demonstrate an effect should not be equated with proving the absence of an effect. As the authors acknowledge, the results of this small study should be interpreted with caution. Appropriate sample size to detect clinically relevant differences and minimize likelihood of type I and type II statistical errors was not achieved. The timing of harvest of the tissue varied from 42 to 374 days after cell injection. Thus, given the high spatiotemporal variation in the vascular niche controlling organ regeneration, potential effects simply may have been missed (13). There are a number of additional limitations to the current trial beyond the small numbers and timing of histopathological evaluation, including cell potency, cell dose, variability in the areas treated, assessment of vascular tissue, as well as the end-stage nature of the disease.

An investigation into the mechanism to explain an observed effect is strengthened by first optimizing and standardizing efficacy. The number and potency of autologous stem cells decline with age and cardiovascular risk factors. In this trial, patients were older, 5 of 6 had diabetes, the percentage of CD34+ cells ranged from 0.6% to 10.8% and there was no measure of cell potency. The major limitation would appear to be the low cell dose: the CD34+ cell dose was 0.5 × 10^6 CD34+, which is 70 to 80 times lower than the effective dose established in rats (4) and 10 to 15 times lower than the dose used in the refractory angina trial (12) based on a 70-kg human. In fact, published data in healthy, young animals have indicated that a dose of <10^5 cells/kg had no measureable effect. Reduced cell efficacy and dramatically reduced doses may very well explain the negative findings reported in this study. Would a 1-mg dose of aspirin be effective in acute coronary syndromes or with percutaneous coronary intervention?

Potency of the cellular product was not tested with respect to its angiogenic potential before initiating the clinical study (14). Unfortunately, without knowing the potency of a therapeutic agent, it is essentially impossible to draw any meaningful conclusions. This is specifically pertinent for the use of “biologics,” which are profoundly affected by inherent patient factors, as is the case for autologous cellular products.

Another potential limitation is the end-stage nature and heterogeneity of ischemic cardiomyopathy. Injection sites were selected based on 50% to 75% perfusion by technetium scan. Location heterogeneity alone might explain any observed differences attributed here to treatment effect given the small sample size especially in combination with the low number of cells and significant delay to histopathological examination. Evidence suggests that the predominant mechanism of benefit is paracrine via the recruitment of endogenous cells. However, the expression of CD31, which was used to characterize endothelial cells, is not limited to vascular tissue, but it is also found on myeloid cells including monocytes and macrophages making interpretation of the observed results even more cumbersome.

Finally, very end-stage ischemic cardiomyopathy may be a particularly challenging environment. In fact, these patients rarely exhibit reverse remodeling with unloading the heart by LVAD implantation.

We are left with the following possibilities: 1) preclinical studies are not predictive; 2) the present clinical model was flawed; or 3) the findings from preclinical studies and the present clinical study are both accurate but the preclinical “mice” models are not relative to “men”?

The current research climate in cell therapy encourages traducing translation. However, each of the explanations—mechanistic differences, sampling error, limitations of the human model—must all be
excluded before we conclude that translation fails. In this case, the “absence of evidence is not evidence of absence” paradigm is not the end of the conundrum but the needle pointing the way to the solution.

**REFERENCES**


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