EDITORIAL COMMENT

A Longer, Better Ride With Engineered Stem Cells*

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A television series called *Pimp My Ride* depicts the transformation of run-down, shabby automobiles into fantasy-lands of extreme color and function customized to the owner's interests, with built-in light shows, clothes dryers, sound studios, and movie theaters. At the end of each episode, we are presented with a dazzlingly transformed, attention-getting vehicle. However, the viewer usually does not see how well the revamped car performs or how long it stays on the road.

The promise of curing heart failure with stem cells has a similar attention-getting dazzle. The vision of regenerating healthy myocardium in a diseased heart has created both hope and skepticism, and clinical deployment of regenerative therapies is continuing apace. The first trials of bone marrow stem cell delivery to the diseased myocardium began in 2001 (1–4); the literature now encompasses hundreds of preclinical studies and dozens of clinical trials. The safety of this approach is well established (5), with promising suggestions of reductions in myocardial infarct size and improvements in exercise tolerance, and comparatively smaller changes in community-accepted functional measures (e.g., left ventricular [LV] ejection fraction [EF]). Results of a recent Phase II study of bone marrow stem cell injection in ischemic heart failure (FOCUS-CCTRN [First Mononuclear Cells Injected in the United States Conducted by the Cardiovascular Cell Therapy Research Network][6]) were negative with respect to primary outcome measures of oxygen consumption and LV end-systolic volume. Exploratory analyses showed a significant, albeit small (2.7% relative to placebo), treatment-related improvement in LVEF associated with treatment. Intriguingly, changes in LVEF were directly related to the proportion of bone marrow cells that express cell surface markers CD31 and/or CD133. These markers identify a specific type of progenitor with the potential to form endothelial and vascular structures (7,8). The finding provides important clinical support for the intuitive notion that the biological properties of cells used for regenerative therapy will be critical for therapeutic success.

An important next step is to identify and optimize those cells that have real therapeutic horsepower. Other cell types with cardiac regeneration potential have now entered the clinic, including a population of resident myocardial progenitors expressing the cell surface marker c-kit (9–11). The recent publication of the Phase I SCIPIO (Cardiac Stem Cell Infusion in Patients With Ischemic Cardiomyopathy) (12) and CADUCEUS (Cardiosphere-Derived Autologous Stem Cells to Reverse Ventricular Dysfunction) (13) trials represent initial proofs of concept and safety for these and related cardiac progenitor cells (CPCs).

The study by Mohsin et al. (14) in this issue of the *Journal* takes a further important step. The authors have customized c-kit+ CPCs with a protein, Pim-1, which equips them with features that enhance their repair potential. Pim-1 is a serine/threonine protein kinase from a 3-member family that shares consensus phosphorylation targets with Akt and regulates many of the same effectors of proliferation, survival, and cell cycling (15,16). The authors previously found that Pim-1 acts as an important effector of myocyte survival downstream of Akt (17). Here they show that Pim-1–expressing cells, compared with control cells expressing only a marker gene, had significant enhancement of a variety of properties associated with regenerative capacity, including increased proliferation, engraftment, differentiation, and persistence in an immuno-compromised mouse model of myocardial infarction. Critically, the authors could demonstrate significant reduction in infarct size and functional improvement associated with Pim-1 stem cells. These results establish the concept that genetic engineering of progenitor cells has the potential to provide the next important advance in regenerative therapy for the heart.

In a way, this study (14) also represents an advance in gene therapy for heart failure. Studies using direct gene transfer to myocardium (e.g., S100A1 [18] and SERCA [sarco/endoplasmic reticulum Ca^{2+}-ATPase] [19,20]) have provided an important conceptual foundation, but gene therapy as an approach remains dogged by theoretical and practical concerns about uncontrolled distribution of the therapeutic vector elsewhere in the body, viral integration, lethal host reactions, and rapid elimination. In the present study, incorporation of a replication-defective vector into the genome of the stem cell seems to solve many of these problems, although it remains possible that expression of genes driven by the viral vector will have unexpected effects on the antigenic properties of

transduced cells that cannot emerge in the NOD/SCID mouse.

Pim-1 differs from many other kinases in that it requires no post-transcriptional modification to become active, and therefore its activity is determined by its levels in the cell (21). Expressed at varying levels in most tissues, Pim-1 can be induced by cytokine stimulation, oxidative stress, and nutrient deficiency and can be thought of as an adaptive response to hostile surroundings. In this regard, Pim-1 is an interesting if not ideal choice as special equipment for cells that need to survive as long as possible in a foreign environment, without a blood supply or native matrix attachments. Cancer cells are expert at this type of survival, and indeed Pim-1 is a proto-oncogene, shown to be a weak tumor promoter when overexpressed alone and a strong tumor promoter in the presence of c-Myc gain of function (22,23). By permitting cells to override normal blocks to mis-timed cell cycling, Pim-1 may enhance genome instability as part of its tumorigenic potential. A further concern is the fact that, in clinical practice, most autologous CPCs will be taken from older individuals with higher numbers of acquired somatic mutations and cancer risk. These issues will need to be addressed in future work. In the present study (14), although Pim-1–engineered cells persisted significantly longer after injection into the myocardium, they nonetheless were undetectable after 8 weeks, reducing concerns about their tumorigenic potential.

Does this prolonged "dwell time" account for the therapeutic advantages of Pim-1 expression? Greater durability could provide more time for the CPCs to exert beneficial paracrine effects on the myocardium, which may be their most important mechanism of action. Remarkably, the Pim-1–transduced human CPCs were not only able to differentiate but seem to have done so more efficiently along myogenic and vasculogenic lines than the unmodified cells (14), suggesting that Pim-1 effects do not present a barrier to cellular maturation. However, given the eventual disappearance of the cells, it seems that whatever differentiation properties they may have are less significant than the fact that they have greater staying power.

A remarkable fact is that the cells used in the present study (14) were obtained and clonally expanded from a single 65-year-old man with heart failure severe enough to warrant mechanical cardiac support. The authors report success in obtaining CPCs from multiple subjects, confirming the feasibility of generating functional CPCs even from patients with advanced heart disease. Given the difficulty and expense of isolating these cells, the creation of a biorepository of such patient CPCs would be a valuable tool for understanding the limits of endogenous regenerative potential, correlating cell function with patient characteristics, and generating new ideas for genetic "customization" based on specific cell properties.

Further studies will no doubt reveal other molecules conferring desirable features, such as cardiogenic or vasculogenic differentiation, long-term viability, or enhanced paracrine activity. Controversies still persist over how progenitor cells actually contribute to benefit, and whether 1 type is to be preferred over others. With the oversized hope attached to stem cells in general, it looks as if many of these disputes will be sorted out initially in human studies, rather than in the laboratory. The study by Mohsin et al. (14) comes not a moment too soon to show us that genetic engineering is not just about flashy body work but a way to a more powerful ride for CPCs as they move into the clinic.

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