The notion of cell transplantation into the heart as a means of reversing ischemic injury is now nearly a decade old. The first clinical application of bone marrow mononuclear cells (BMCs) for myocardial infarction was in 2001, presumably motivated at least in part by the premise that BMCs injected into the heart can directly regenerate new, functional myocardium. Although subsequent investigators questioned the ability of BMCs to transdifferentiate into cardiomyocytes (but in all fairness, others did support the idea), the horse was out of the barn, and the treatments continued apace. Fortunately, injection of autologous BMCs as adjunctive treatment for convalescent myocardial infarction has proven to be remarkably safe. Although the overall efficacy is modest, certain subgroups (particularly those with large functional deficits at baseline) do experience clinically meaningful increments in ejection fraction. A related consideration arises in the choice of cell type to transplant. BMCs, although easy to harvest, are almost certainly not the best candidate cells for cardiomyoplasty, because they are not specialized to regrow normal healthy heart muscle. An attractive alternative arose with the recognition that the adult heart contains its own reservoir of progenitor cells, some of which express the stem cell antigen c-kit. Such cardiac progenitor cells (CPCs) presumably function physiologically to mediate a low basal turnover rate of cardiomyocytes in the adult heart but may be expanded and exploited iatrogenically for more focused (and, mechanistically, more rational) benefit than may be possible with BMCs. When injected into the injured heart, CPCs increase tissue viability and improve ventricular function.

Ironically, we are now less certain about the mechanism of cell therapy than we thought we were a decade ago, when it seemed that direct regeneration of tissue from transplanted cells was the whole story. Several new questions are now front and center. Because long-term cell-engraftment rates tend to be very low, direct differentiation into a cardiovascular phenotype may be insufficient to explain the positive outcomes. The alternative explanation, the “paracrine hypothesis,” posits that transplanted cells effect much if not all of their benefit by tissue preservation and/or recruitment of endogenous regeneration. The first question thus becomes, what is the relative role of direct regeneration (through formation of new tissue) versus indirect mechanisms (through paracrine effects) in mediating the functional benefits of cell transplantation? Second, in the scope of direct regeneration, what is the functional relevance of stem cell fate in terms of lineage? We recognize that both cardiomyocytes and vascular elements are necessary for regrowing healthy heart muscle, but the relative importance of cardiomyogenesis versus angiogenesis in functional recovery is unknown. Third, what is the role of CPCs in general, and c-kit+ CPCs in particular, in cardiovascular regeneration? When injected into the injured myocardium, does the amount and potency of c-kit positivity trump other considerations in dictating the outcomes of cell therapy?

Two articles in this issue of Circulation address the questions enumerated above. Yoon et al made the first attempt to elucidate the functional contribution of the lineage commitment of human BMCs in vivo. In the second study, Zaruba et al investigate the cardiomyogenic potential of c-kit+ cells derived from neonatal and adult mouse hearts.

In the first study, Yoon et al engineered viral vectors encoding inducible suicide genes under the control of endothelial (endothelial nitric oxide synthase)-, smooth muscle (SM22alpha)-, and cardiomyocyte (alpha-myosin heavy chain)-specific promoters. This strategy enabled selective depletion of the individual cell lineage acquired by the transplanted undifferentiated BMCs. The striking finding is that elimination of transplanted endothelium-committed or SM22alpha-expressing cells, but not cardiac-committed cells, induced a significant deterioration of heart function (ejection fraction). The data also suggested a strong correlation between vascular density and ejection fraction. Two important messages of these data are that (1) the mechanism underlying the functional benefit of BMC therapy preferentially involves stem cell–mediated angiogenesis but not cardiomyogenesis, and (2) the early-stage beneficial effects of paracrine factors could be largely diminished by later elimination of a single lineage (in this case, endothelium-committed cells). The critical role of vascular differentiation for BMC therapy is not entirely surprising, because numerous lines of evidence suggest that BMCs target vasculature but not myocardium. Previous studies have demonstrated that transplantation of unpurified BMCs improved blood flow recovery and capillary density after ischemia. The irrelevance of cardiomyogenesis to functional preservation is possibly due to the very small number of cardiac-committed cells in BMCs (harking back to the historical notes in the first paragraph).
Although Yoon et al\textsuperscript{23} performed careful examination of suicide induction on neighboring cells (so-called bystander effects), this possibility remains an intrinsic flaw of the thymidine kinase/ganciclovir system. The toxic byproducts of thymidine kinase/ganciclovir in the cytosol are sufficiently small that they can cross from cell to coupled cell via gap junctions, thus potentially overestimating the contribution of direct regeneration. Negative results cannot be rationalized away, but positive results may be exaggerated. Although it represents the most widely used suicide gene, the thymidine kinase/ganciclovir system has a number of other drawbacks, including its immunogenicity and its effects on cell division. Alternative suicide genes such as inducible caspase 9 merit future investigation.\textsuperscript{26,27} Nevertheless, the study by Yoon et al\textsuperscript{23} provides in-depth mechanic insights into the relative role of cardiomyogenic lineage commitment of injected human BMCs for cardiovascular regeneration.

As pointed out by Yoon et al\textsuperscript{23}, a major role for paracrine effects could not be excluded. Although the functional benefit of BMC translation appears to be strongly correlated to the generation of new vasculature, BMCs or BMC-derived endothelial cells can also promote angiogenesis and possibly cardiomyogenesis by releasing paracrine factors.\textsuperscript{28} It is unclear whether the deterioration of heart function was due to the depletion of direct vascular regeneration by endothelial nitric oxide synthase–expressing cells or due to the depletion of paracrine factors secreted by the same population of cells, or whether it was overestimated owing to the bystander effect of the thymidine kinase system.

In the second study in this issue of Circulation, Zaruba et al\textsuperscript{24} challenged the existing paradigm that c-kit\textsuperscript{+} resident CPCs in the adult heart can readily differentiate into a cardiomyocyte phenotype. The authors examined the cardiomyogenic potential of c-kit\textsuperscript{+} cells isolated from normal neonatal, normal adult, and infarcted adult mouse hearts. Zaruba and colleagues\textsuperscript{24} detected apparent cardiomyogenic activity in the c-kit\textsuperscript{+}/CD45\textsuperscript{−} subpopulation of cells from neonatal hearts but not the c-kit\textsuperscript{+}/CD45\textsuperscript{+} or c-kit\textsuperscript{−}/CD45\textsuperscript{+} cells from the bone marrow. This is consistent with the study by Yoon et al\textsuperscript{23} because the BMCs in that study had very limited cardiomyogenic potential. The most surprising finding by Zaruba et al\textsuperscript{24} is that c-kit\textsuperscript{+} cells from normal adult hearts failed to undergo cardiomyogenic differentiation when cocultured with fetal cardiomyocytes or when transplanted into normal or infarcted adult mouse hearts. These data suggested that the ability of cardiac-resident c-kit\textsuperscript{+} cells to acquire a cardiomyogenic phenotype is limited or that the cardiomyogenic population is lost during development. These results differ from those of multiple previous studies using adult heart–derived c-kit\textsuperscript{+} cells from rat and human hearts. There, coculture of CPCs with cardiomyocytes or injection of those cells into injured myocardium resulted in much more robust cardiomyogenic differentiation.\textsuperscript{12,29} The origin of the discrepancies is unclear, but it is noteworthy that Zaruba et al\textsuperscript{24} found much higher percentages of c-kit\textsuperscript{+} cells in the bone marrow (9%) and in the heart (0.5%) than have been reported previously (eg, 0.0025% in the heart in a report by Beltrami et al\textsuperscript{12}). Others have found that c-kit is critical for the conversion of hematopoietic stem cells to cardiomyocytes.\textsuperscript{6} So, even if some of the c-kit\textsuperscript{+} heart-derived cells in the present study by Zaruba et al\textsuperscript{24} had been populated in the heart by hematologic seeding, it is unclear why their cardiomyogenic potential was so limited. On the other hand, some of the same authors who had argued for robust cardiomyogenesis with c-kit\textsuperscript{+} CPC transplantation now report that direct cardiac regeneration from the transplanted cells is rare, despite durable morphological and functional benefits.\textsuperscript{30}

The emerging picture is confusing for purified c-kit\textsuperscript{+} CPCs. Perhaps such cells are indeed relatively unimportant; alternatively, their potency may be artificially minimized when they are antigenically selected and transplanted in isolation rather than in the context of a supportive mixed-cell milieu. Over the past 6 years, we have been developing human cardiosphere-derived cells (CDCs) grown from percutaneous endomyocardial biopsy samples as a potential therapeutic product. CDCs are a natural mix of heart-derived cell subpopulations, including c-kit\textsuperscript{+}/CD90\textsuperscript{−} CPCs and cardiac mesenchymal stem cells (c-kit\textsuperscript{−}/CD90\textsuperscript{−}).\textsuperscript{30,31–33} We have found that unsorted human CDCs are functionally more potent than c-kit\textsuperscript{+} or CD90\textsuperscript{+} purified stem cell subsets.\textsuperscript{34} In studies with intramyocardial injection of human CDCs in a murine model of myocardial infarction, the unselected CDC mixture resulted in a higher ejection fraction at 3 weeks than either purified c-kit\textsuperscript{+} or CD90\textsuperscript{+} cells from the same source. These findings imply that cardiac mesenchymal stem cells help CPCs, perhaps fostering the synergistic effects of favorable paracrine effects and direct contributions to myocardial regeneration. Along these lines, we have found that both direct regeneration and paracrine effects contribute to the therapeutic benefit of CDC transplantation, with the latter as the predominant factor.\textsuperscript{18} On the basis of the number of human-specific cells relative to overall increases in capillary density and myocardial viability, we found that direct differentiation quantitatively accounted for 20% to 50% of the observed effects.

CDCs (CADUCEUS [Cardiosphere-Derived Autologous Stem Cells to Reverse Ventricular Dysfunction] trial) and purified c-kit\textsuperscript{+} CPCs (SCIPIO [Cardiac Stem Cell Infusion in Patients With Ischemic Cardiomyopathy] trial) are already in phase I or II clinical trials. If, indeed, heart-derived cells (antigenically selected CPCs or mixed CDCs) exert most of their benefits via indirect mechanisms, are they superior to BMCs or to bone marrow–derived mesenchymal stem cells, which appear also to work predominantly via paracrine effects? To settle this question, direct head-to-head comparisons of the various cell types, in the same model, will be required, along with quantification of the secretion of relevant growth factors. Laboratories are often reluctant to perform such unglamorous and difficult experiments, preferring instead to use their own favorite cell type, but such comparative studies are absolutely essential to the progress of our field. We do know that CDCs are rich biological factories capable of producing large amounts of myriad secreted factors.\textsuperscript{18} Whether they will be superior to other cell types in this regard remains to be determined.

The heart is complicated; so is heart regeneration. The elusive mechanisms underlying functional improvement include angiogenesis, cardiomyogenesis, extracellular remod-
eling, paracrine factors, recruitment of stem cells from within and outside the heart, dedifferentiation, and myocyte cell cycle reentry. All of these processes can plausibly be affected by injected cells, directly or indirectly. Although the road has twists and turns, the future of regenerative cardiology is wide open. What we must not allow ourselves to do is to be paralyzed by the immensity of our own ignorance. As long as the risks are small relative to the potential benefits, and the appropriate preclinical studies have been performed, innovative clinical trials cannot be considered premature, particularly in sick patients with few other options. Here, the words of Ivan Turgenev are relevant: “If we wait for the moment when everything, absolutely everything, is ready, we shall never begin.”

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