activates apoptosis by plasma membrane death receptor pathway, activation of caspase enzymes, and release of cytochrome c from mitochondria. Although blood flow to the spinal cord is restored during reperfusion, the motor neurons that seem to survive ischemic insult may undergo delayed selective death, particularly 7 days after the procedure.⁴ Despite that the etiology of delayed selective neuronal death has been proposed to be the activation of Akt protein, Grp78, and caspase 12 proteins, further studies should be warranted. In the present study, the authors claimed the protective effect of edaravone on spinal cord injury according to the histologic examination and levels of ROS. However, it is known that these neurons might be apoptotic despite the normal cellular architecture seen on the hematoxylin and eosin (H & E) staining. The authors did not focus on apoptotic mechanisms and demonstrated only necrotic (ghost) neurons with H & E staining without considering the neurons undergoing apoptosis. In this regard, we believe that addition of the neuronal apoptosis would increase the statistical power of this study.

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doi:10.1016/j.jtcvs.2005.12.056

Reply to the Editor:

We thank Dr AK for his insightful comments on our recently published article. Paraplegia or paraparesis occurring in the late postoperative period is well described in the literature. The mechanism of this delayed spinal deficit is presumed to be related with apoptosis. Sakurai and colleagues¹ evaluated the relationship between delayed paraplegia and apoptosis using immunohistochemical techniques. In their experimental study, the transient spinal ischemic time was 15 minutes. Two days after the reperfusion, the mean Johnson score was 4.9 ± 0.894 , and 3 of the 5 rabbits had normal hind-limb function. In our study,² we applied a spinal ischemic time of 30 minutes. Two days after the reperfusion, all rabbits in the control group were completely paraplegic with a Johnson score of 0. The focus of our investigation was to assess whether prophylactic administration of edaravone could suppress necrosis and not apoptosis of the spinal cord. Consequently, a longer spinal ischemic time was employed and only hematoxylin-eosin staining was used for histopathologic evaluation. As suggested by AK, motor neurons that appear intact on hematoxylin and eosin staining as a result of the prophylactic administration of edaravone might suffer delayed apoptosis-related injury. Because we did not use any marker for apoptosis in our study, we are not in a position to comment on this. However, we agree with Dr AK that further studies need to be undertaken to clarify this issue. We are indeed planning to embark on a protocol that involves the use of markers of apoptosis in a model of shorter spinal ischemic time and longer duration of observation.

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Surgical treatment for congestive heart failure with autologous adult stem cell transplantation

To the Editor:

Patel and colleagues¹ have provided further evidence of the benefit of autologous stem cell transplantation in patients with ischemic cardiomyopathy. However, even if, in the past years, observational studies and some randomized controlled trials have established that autologous stem cell transplantation has led to significant improvement in myocardial infarction and congestive heart failure, this study unfortunately has not specifically addressed several clinically relevant questions: What dose of CD34+ cells for stem cell therapy in this area should we use? Furthermore, what is the best cell type and the best cell dose for each cell type?

In recent studies, doses of CD34+ cells ranging from 10^6 to 10^8 have been used; however, if the optimum dose of CD34+ cells needed is proven to be much less than 10^6 , procedures involved in collecting sufficient amounts for therapeutic use can be less time-consuming and thus potentially cost-saving. Furthermore, the expression of CD34 surface antigen characterizes a heterogeneous population of cells including hematopoietic progenitor cells, endothelial progenitor cells, mature endothelial cells, and tissue-committed stem cells as recently reported.² It is still not clear whether the beneficial effect of these cells in regeneration can be explained by the transdifferentiation of hematopoietic stem cells, the paracrine secretion of angiopoietic factors from bone marrow-derived stem cells,³ or the presence of tissue-committed stem cells for myocardium or endothelium. We believe that there is a pressing need to standardize therapeutic protocols to allow tailor-made therapies for these patients.

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doi:10.1016/j.jtcvs.2005.12.048

Reply to the Editor:

I thank Dr Vincenzo Pitini for his comments and concerns regarding our recently published article.¹ The salient questions regarding the optimal cell type, dose, purity, method of injection, and final fate of the cells are all important and need to be addressed. However, this article was the first randomized study to address some of the more basic issues related to the use of cell therapy in an adjunctive fashion to revascularization. Because there is a chance of overlap between collateral vessels and regions of cell injection that were not directly revascularized, we wanted to see whether there was a difference over and above revascularization. This was found to be true. To address the other questions I will go in order. First, cell type along with purity was thought to be very important. The basic and

preclinical models using various fractions of blood or bone marrow, adipose, myoblasts, umbilical cord, or amniotic cells have all shown promise of cardiac differentiation. However, myoblasts, blood, and bone marrow have been used clinically showing early promising results; only myoblasts have problems related to ventricular arrhythmias. Both blood and bone marrow have been used as a relatively pure formulation of CD 34+, AC 133+, mesenchymal cells, or a mixed population based on density gradients. Neither has truly shown a benefit over the other thus far. Therefore, no one really knows the optimal cell type or purity yet. As to the dose of cells, according to the work of Stamm and colleagues² on AC 133+, there has been no dose-response curve.² Currently, at the University of Pittsburgh, we have a Food and Drug Administration-approved Phase I prospective randomized blinded trial for 3 arms of low, medium, and high doses of CD 34+ and 1 placebo arm for coronary artery bypass grafting and cells for patients with ejection fractions of less than 40%. This may help lead to more answers at least for this cell type. However, dosing trials will be needed for each different cell type used and each clinical indication desired. The method of injection is also a variable related to success of delivery and possible retention of cells in the tissue. We have been using a modified needle with side holes injected on an angle and left in the tissue for a short period of time to decrease leakage. This has been successful, but the use of biodegradable gels is promising but not yet clinically applicable. We have also started partnering with industry to develop an automated cell injection sys-

tem to help increase the reliability of cell delivery in terms of accurate doses. Finally, the fate of the cells has led to many proposed mechanisms from differentiation, to fusion, to a paracrine response. We do not have the answers yet in humans, but we do have a Food and Drug Administration-approved trial for the CD 34+ cells injected into the hearts of patients with a ventricular assist device implanted as a bridge to transplant. Half of the heart is injected with cells, and the other half is injected with a placebo. The heart is explanted at the time of transplantation, and the tissue is analyzed for cellular changes. Again, the issue arises because the cells cannot be marked and tracked once in vivo. Overall, the early clinical trials are promising, but there is a lot of research that still needs to be performed to help understand the clinical improvements seen in our and many other patients.

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