Cell Therapy for Stroke
Remaining Issues to Address Before Embarking on Clinical Trials

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Background and Purpose—Stroke remains a significant clinical unmet condition, with only 3% of ischemic patient population benefiting from the thrombolytic drug tissue plasminogen activator largely because of the drug’s narrow 3-hour therapeutic window. Extending the stroke therapeutic window will greatly impact on treatment, care, and management of patients.

Summary of Review—Cell therapy is appealing in this regard as it widens the stroke treatment opportunity by targeting the neurorestorative phase (ie, several hours to days and even weeks or months after stroke). Although compelling preclinical evidence reveals that transplantation of stem/progenitor cells is safe and effective in animal models of stroke, the laboratory data need to be evaluated on their translational relevance for clinical application. In addressing this issue, I borrow heavily from the conference proceedings of the 2007 STEPS (Stem Cell Therapeutics as an Emerging Paradigm in Stroke).

Conclusions—Translational research guidelines are being adapted by academic institutes, industry, National Institutes of Health (NIH), and Food and Drug Administration (FDA), and adhering to these preclinical criteria will provide the basis for advancing cell therapy in stroke from the laboratory to the clinic. (Stroke. 2009;40[suppl 1]:S146-S148.)

Key Words: cell transplantation ■ cerebral ischemia ■ translational medicine

Are we there yet? The miserable track record of neuroprotective drugs in the clinic, despite positive outcome in the laboratory, suggests that the preclinical data do not closely approximate clinical outcome. This obvious disconnect between the laboratory and the clinic has prompted us to revisit our laboratory experience with cell therapy and stroke. First, I will discuss critical issues that hinder the translation of cell therapeutics into clinical application can be categorized into academic/industry and FDA gating items. Second, I will propose a resolution to enhance the success of translating cell therapy in stroke from bench to bedside.

Academic/Industry Issues

How Close Are Animal Models to Human Stroke? Clinicians recognize that no two strokes are the same, underscoring the heterogeneous pathophysiology of the disease. Although the target disease of cell therapy is ischemic over hemorrhagic, still ischemic stroke is plagued with several subtypes, including differences in brain infarcted areas or cell death mechanisms. Laboratory investigations have only recently attempted to encompass these different strokes in animal models. Until now, the most widely used model is a focal ischemia using rats or mice. The damaged brain regions and cell death pathways after focal ischemia can be manipulated by using transient, permanent, distal, or proximal middle cerebral artery occlusion/ligation. To capture the heterogeneity of stroke, STEPS recommends the use of at least 2 focal ischemia models, in addition to multiple strains, age, and gender to test the efficacy and safety of cell therapy. Moreover, as the nonhuman primate (NHP) model of stroke becomes more established, testing cell therapy in this larger animal model is needed, primarily because the white matter injury associated with stroke is not well developed in the rodent model. The white matter injury including alterations in the neurovascular unit can be replicated in the NHP accompanied by stable motor and cognitive deficits, although neural plasticity especially in the cortex may attenuate specific behavioral deficits.

Why Is There a Need for Donor Cells to be ID’ed? For testing efficacy and safety, the donor cells need to be initially characterized in vitro using phenotypic expression assays among other markers to allow quality control and assurance that a well-defined cell population is generated, maintained, and banked as transplantable cells. This same set of phenotypic markers can be used to track the survival, migration, and differentiation of the cells after transplantation. The parameters for cell proliferation, especially if the donor cells are genetically manipulated, should be assessed to determine the need for regulating uncontrolled cell division which could be a cause for tumorigenesis and ectopic tissue formation.
Can I Use My Favorite Behavioral Test?
In characterizing the behavioral deficits in animals after ischemic stroke, the guiding principle is to faithfully mimic the clinical symptoms. A battery of behavioral tests will likely allow a closer approximation of human correlates of functional dysfunctions, and these tests should also be used to monitor the behavioral recovery after cell transplantation. Motor and somatosensory deficits are well documented in ischemic rodents, and there is also a trend toward assessment of cognitive deficits. However, alterations in cognitive behaviors can only be realized when cognitive brain areas are damaged by stroke, thus not all ischemic stroke animals, in particular those with very localized damaged sparing the hippocampus or specific regions of the basal ganglia, should be subjected to cognitive testing. A long-term (ie, at least one month) testing should also be performed to reveal whether the cell transplant–mediated functional recovery is stable or temporary, with the latter suggesting a repeated dosing regimen for sustained therapeutic benefit.

To facilitate reproducibility, replication, and validation of the data, the procedures for animal modeling, cell isolation, and maintenance, including information on phenotypic markers, should be freely shared with other investigators (of course after intellectual property disclosures and the like have been completed).

What Experimental Design Should I Follow?
The research design should closely approximate the envisioned clinical application. Optimization of the cell dose, delivery route, and timing of administration are the 3 most important factors that need to be determined in the laboratory. Based on the optimization results, the next critical goal is to reveal whether the cell dose, route, and timing are clinically practical and feasible. Clearly if the cell dose of 1 billion cells was shown to promote behavioral recovery and the cell production does not allow such ample cell supply, then further testing in the clinic is not indicated. In the same vein, if stereotaxic delivery and transplanting the cells immediately after stroke (within hours after stroke onset) were found prerequisite for therapeutic benefits, then such intervention obviously limits application to only a small number of patients. With these preclinical go, no-go criteria, the clinical research design should completely adhere to the laboratory data which may indicate a careful selection of stroke patients who will likely benefit from cell therapy in addition to closely extrapolating the dose, route, and timing of cell transplantation.

For Translational Research, Is There Room for Mechanistic Investigations?
To date, the mechanisms underlying cell therapy–mediated functional recovery remains not fully understood. Should we proceed to the clinic without knowing the factors mitigating the functional effects of cell therapy? Both translational and mechanism-driven experiments can simultaneously be pursued. Once efficacy and safety of cell therapy are validated, limited clinical trials may be considered, while at the same time investigations into the mechanisms should continue to be explored because identifying the pathways for cell restoration can be subsequently used to further improve the functional benefits of cell therapy. Cell signaling and growth pathways, in tandem with postulated neurorestorative processes such as neurogenesis, angiogenesis, synaptogenesis, immunomodulation, trophic factor secretion, and cell replacement, if proven to mediate neurorestoration, are likely to become effective targets for adjunctive treatments for cell therapy. Imaging techniques, such as in vivo functional MRI, have visualized both graft and host response in stroke models, and are poised to become vital tool for mechanistic investigation underlying cell therapy.

FDA Issues
I acknowledge that the succeeding views are my own interpretations of the FDA opinion about cell therapy in stroke.

Safety First: Are Transplanted Cells Safe?
The FDA resonates Hippocrates’ creed of “first do no harm” to the patient in evaluating the entry of cell therapy into the clinic. Here, the FDA requires proof that transplanted cells do not form tumors or ectopic tissues, that they do not produce overt behavioral abnormalities, and that they do cause overt physiological alterations. The safety outcome should be evaluated in the animal model that closely mimics the clinical condition. In this case, if an allogeneic transplantation is envisioned, then an allogeneic transplant paradigm should be tested in the laboratory, thereby requiring transplantation of rat cells into rat models then determining any tumor formation, and behavioral and physiological changes. For genetically manipulated cells, the recent stance of the FDA is to monitor the transplant recipients over its lifespan. Finally, the need for assessment of not only tolerability but also toxicity requires testing of higher cell doses beyond threshold or therapeutic doses. Taken together, these safety outcome measures are designed to exclude tumorigenicity.

Efficacy Next: Is Dose Response Study Necessary?
The rationale for pursuing a dose–response study is not only to fully characterize the cell therapy benefits by demonstrating the most efficacious dose, but also to determine the tolerable dose, both of which will aid in extrapolating the clinically relevant cell dose. However, this is a point of contention where the FDA welcomes a solid justification on how to extrapolate laboratory dose to clinical dose. Applications for IND on cell therapy have alluded to comparing rodent and human brain weight, size, or stroke volume in clinical derivation of the cell dose obtained from animal studies. Additional studies are warranted on unequivocally determining the clinically relevant dose.

Additional Safety Concern: Oh Where, Oh Where Are My Cells?
The FDA has taken a keen interest in the mechanism of action of transplanted cells, in particular as noted above the neurorestorative pathway afforded by genetically manipulated cells. The shift in mechanistic concepts from “cell replacement” to “trophic factor secretion” in graft-mediated behavioral recovery is being recognized by the FDA. To this end, whereas long-term cell persistence seems not necessary for a trophic factor–mediated behavioral recovery, the FDA right-
fully asks about the status of the cells: where are they deposited? What is their eventual fate? If there is no evidence of cell persistence in the brain or the periphery, how are the grafted cells disposed from the organism? In case of genetically manipulated cells, do they get transformed? Although these questions appear as mechanism-driven, which may very well be, the FDA views them as pertaining to a highly relevant safety concern. For example, if cells did not get disposed from the organism and were transformed into dormant but can achieve a proliferative state when stimulated, over time these cells may be a source of tumors. Accordingly, there is a need to assess the eventual fate of grafted cells.

Proposed Resolution
What Is the Missing Translational Step?
Recently the NIH National Institute on Neurological Disorders and Stroke solicited grant applications for the establishment of Preclinical Stroke Consortia that would create an alliance among academic institutes and industry under the guidance of NIH and FDA tasked to translate therapeutic interventions from the laboratory to the clinic. In response to this call, we formed a Preclinical STEPS Consortium consisting of 3 laboratory stroke–cell therapy experts including my laboratory at Medical College of Georgia, Henry Ford Hospital (Dr Michael Chopp), and Stanford University (Dr Gary Steinberg), working closely with stroke–cell transplant clinician experts (Dr David Hess, at Medical College of Georgia, and Dr Douglas Kondziolka, University of Pittsburgh); these laboratory-clinic components are interfaced via a Data Coordination Center (Dr Mei Lu, Henry Ford Hospital). Our overarching goal is to test the safety and efficacy of the same donor cells in the same stroke models, and using the same standardized tests in multiple laboratories, guided by the STEPS criteria,2,3 as well as the academic/industry and FDA concerns which I discussed above. Our consortium recognizes that to date there currently exists no multiple laboratory testing of cell therapy in stroke. Henceforth, we advance the unique approach of validation, reproducibility, and replicability of safety and efficacy of cell therapy in stroke via a Preclinical STEPS Consortium.

Conclusion
In conclusion, a number of critical safety and efficacy issues remain, which may be best approached by a coordinated effort across multiple laboratories working together under NIH and FDA guidance. The use of the STEPS guidelines provides a stringent evaluation of translating cell therapy for stroke from the laboratory to the clinic.

Acknowledgments
I thank Drs Michael Chopp, Gary Steinberg, Mei Lu, Douglas Kondziolka, and David Hess for intellectual discussions on the subject of cell therapy and stroke.

Sources of Funding
C.V.B. is funded by NIH NINDS grants 1U01NS055914-01 and 2R42NS055606-02, and stem cell-stroke research grants from Athersys Inc, SanBio Inc, and Celgene Cellular Therapeutics Inc.

Disclosures
C.V.B. is a consultant to SanBio Inc and Sanerone-CEL Therapeutics Inc.

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*Stroke*. 2009;40:S146-S148; originally published online December 8, 2008; doi: 10.1161/STROKEAHA.108.533091

*Stroke* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2008 American Heart Association, Inc. All rights reserved.
Print ISSN: 0039-2499. Online ISSN: 1524-4628

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://stroke.ahajournals.org/content/40/3_suppl_1/S146