Cell Transplantation, Vol. 18, pp. 691–693, 2009

0963-6897/09 $90.00 + .00
DOI: 10.3727/096368909X470883
E-ISSN 1555-3892
www.cognizantcommunication.com

Commentary

Who's in Favor of Translational Cell Therapy for Stroke: STEPS Forward Please?

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A consortium of translational stem cell and stroke experts from multiple academic institutes and biotechnology companies, under the guidance of the government (FDA/NIH), is missing. Here, we build a case for the establishment of this consortium if cell therapy for stroke is to advance from the laboratory to the clinic.

Key words: Stem cell transplantation; Tissue regeneration; Cellular therapy; Clinical translation

The Stroke Therapy Academic Industry Round table (STAIR) criteria serve as a guide for translation of drug development programs in an attempt to improve the success of clinical trials of neuroprotective drugs. However, a preclinical stroke consortium approach remains to be created in drug development programs that will include an active collaboration among academia, the pharmaceutical industry, FDA, and NIH. Recognizing that such a vital translational gap between the laboratory and the clinic also plagues the neurorestorative cell therapy program in stroke, we formed the Preclinical STEPS (Stem cell Therapeutics as an Emerging Paradigm in Stroke) Consortium consisting of three preclinical stem cell-stroke expert laboratories interfaced with a data coordination center and guided by a clinical advisory board. Our consortium represents a prototype patterned after NIH's recent RFA on the establishment of Preclinical Stroke Consortia, specifically advancing the novel concept of a multiple preclinical stroke laboratory testing of the efficacy and safety of stem/progenitor cells that echoes both STEPS and STAIR translational crux.

As we solicit the participation of the FDA and NIH in our consortium, we outline below the major goals of the STEPS consortium that are deemed critical to move forward the theme of translational cell therapy in stroke. A major translational research question is determining the "go, no-go" criteria to declare whether cell therapy is efficacious in stroke. If we set the bar too low, would this behavioral recovery in an animal model of stroke translate into clinically relevant functional improvement? Similarly, if we set the bar too high, we may be eliminating the potential of a cell-based therapy to provide small and incremental, but meaningful, daily life activity to the patient. Our position is that cell therapy does not equate to a magic bullet, but we must detect amelioration of experimentally stroke-induced behavioral deficits that have significant clinical relevance. Based on our experience in transplanting bone marrow cells in stroke animals, a significant behavioral improvement was detected based on each individual behavior test score and the observed relative improvement was consistently over than the 25% criterion. Moreover, a review of the literature reveals that the 25% level or better is consistently used as the criterion of behavioral

Received December 18, 2008; final acceptance February 24, 2009.
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recovery from stroke (1–4). Our previous communications with the FDA and NIH support this level of behavioral recovery. These observations formed the basis for employing a 25% or better behavioral improvement criterion. We note, however, that a battery of tests over a single test provides a more solid characterization of behavioral recovery, but this presents as an additional gating item in that the number of tests reaching the 25% criterion needs to be factored in determining a positive or negative outcome. A composite score, similar to an NIH stroke rating scale, incorporating the scores from each task, may aid in interpreting the results from a battery of tests.

An equally translational research criterion is the need for evaluation of cell therapy in at least two species, namely mice and rats, which completely adheres to STAIR and STEPS guidelines. But are mice and rats different enough to be considered different species? If we apply the definition of *species* as a group of related individuals or populations that are potentially capable of interbreeding and producing fertile offspring, then the conclusion is that mice and rats belong to different species as they cannot interbreed. Additionally, mice and rats exhibit major differences in genetic, reproductive, developmental, morphological, and anatomical features. Of particular interest is that rats and mice can exhibit different inflammatory responses to the same stimuli, which could have significant effects on the ability of cell transplantation to work in the two species. For example, the pathogen/cell killing potential of the complement protein complex C5b-9 is very different in rats and mice (5), which could have significant consequences on the survival and function of the transplanted cells. Assuming that the two species only differ slightly, the alternative option may be to pursue a nonhuman primate (NHP) stroke model. Indeed, such testing of cell therapy in NHP was given much consideration in STEPS, but the consensus from these proceedings is a resounding conclusion that to date the NHP model remains to be validated for cell therapy and thus is not appropriate at this time for the present proposal. The use of normal, as opposed to stroke, monkeys may be a feasible alternative, in that one can do a straight toxicology study. However, while such approach will provide partial "safety" and "toxicology" issues, the obvious difference between stroke and nonstroke "normal" pathology will remain a major hurdle in providing insights on stroke pathology and stem cell functionality. Accordingly, validation of NHP stroke model is required prior to employing this animal model for testing cell therapy. Of note, a new NINDS RFA is forthcoming that solicits grant applications for the development of an NHP stroke model. Stroke validation studies in other large animals, such as sheep and dogs, in parallel with the NHP will help bridge the gap between laboratory data in rodents and human applications.

In parallel to demonstrating safety and efficacy of cell therapy in two species is the requirement for testing the experimental treatment in two stroke models. The prevailing notion in animal stroke modeling is that the pMCAo model stands as "a more stringent stroke model" than the tMCAo model. Opponents of this view have raised valid questions. First, it is not at all clear whether humans suffer a form of stroke similar to permanent nylon filament MCAo. It is clear that there is residual flow around all human strokes other than lacunes. Along this line of argument, human stroke is always a combination of total and partial flow arrest. Second, most large vessel occlusions recanalize with or without exogenously supplied rt-PA. Thus, if cell transplantation would target a delayed poststroke time frame (days, weeks, or months after injury), most humans would have reperfused. Third, other than satisfying arbitrary, unvalidated sets of Delphi panel guidelines, the rationale for pursuing both tMCAo and pMCAo models would appear not fully justified. Although many panels have written that tMCAo models could/should be complemented with pMCAo models, there is no validation of this statement. The preceding concerns resonate a longstanding issue about limitations of animal modeling in stroke. Our consortium views that the stroke model needs to focus on focal ischemia, including cortical and cortical plus basal ganglia stroke models. Emphasis is given on clearly delineating the focal brain region damaged by stroke, which will be the basis for choosing the appropriate behavioral tests. Although different types of surgical approaches are available, the "end-point" rather than the "technique employed" is deemed critical in producing the stroke in that the resulting pathophysiologic manifestations of each stroke model should mimic the human disease condition. The pMCAO approach is chosen to provide a model with a severe injury, more severe than the tMCAO model. It should be pointed out that even in a suture pMCAO model there is some collateral blood flow that gets into the infarct. The blood flow is not zero as blood is supplied via collaterals. While the approach to complement tMCAO with pMCAO has not been validated, one would then have to argue that all preclinical work suffers from the same limitation as we still do not have validation of the STAIR and STEPS criteria. Nevertheless, expert and consensus panels have come up with these guidelines and our research team is following these guidelines until we have better validation. The proposed strategy in our consortium to pursue such pMCAo model to complement the tMCAo model will provide the validation of this approach. Of note, a

significantly higher upregulation of chemoattractant-like molecules acutely ensues with reperfusion (6), suggesting tMCAO differs from pMCAo in consequences both for inflammation and targeting of stem cells to the site of injury.

In conclusion, the establishment of a consortium of world leaders in translational stroke-cell therapy research is in line with NIH translational research roadmap, an initiative epitomized in NINDS RFA on Preclinical Stroke Consortia. The STEPS consortium is distinct from drug-based neuroprotective consortia, as our target is the subacute to chronic phase of the disease. With compelling scientific evidence supporting neurorestoration, the field of cell-based therapy deserves testing in a translational setting that will bring this experimental treatment closer to clinical application.

ACKNOWLEDGMENTS: C.V.B. and D.C.H. are funded by NIH 1U01NS055914-01 and NIH 2R42NS055606-02; C.V.B., D.K., and G.K.S. are Consultants to SanBio, Inc.; D.C.H. is supported by NIH/NINDS R01 NS055728; M.C. is funded by NINDS Grants P01 NS23393, P01 NS42345, R01NS38292, P01-NS042345, and R01HL 64766, and is a Consultant to Theradigm, Inc.; G.K.S. is funded by NIH NINDS grants R01 NS27292, R01 NS058784, and P01 NS37520, and is a stockholder in Cardium Therapeutics and has stock options in Stem Cells, Inc., and Neurofluidics. All authors contributed in the conception, writing, and final approval of manuscript.

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