

Stem Cell Therapies as an Emerging Paradigm in Stroke (STEPS)

Bridging Basic and Clinical Science for Cellular and Neurogenic Factor Therapy in Treating Stroke

The STEPS Participants*

Abstract—Investigators developing cellular therapy for stroke face many challenges. Preclinical models used for cellular therapy studies should be relevant to human stroke and predictive of benefit despite differences in stroke size, cerebrovascular anatomy, immune status, and neurological responses. Translating preclinical testing to human trials is compounded by consideration of delivery method and translation of dosing with cell survival. Many issues must be approached in designing clinical trials of cellular therapy for stroke, including appropriate outcome measures, controlling for confounding factors such as rehabilitation therapy, and possible surrogate outcomes using imaging such as MRI and newer imaging techniques. It is also important to establish standardized clinical protocols and clinical database registries in advance of early proof-of-concept studies. Investigators must adopt a standardized nomenclature and characterization schema for cell products to accurately define potency and determine clinical outcome from early proof-of-concept studies. The Stem Cell Therapies as an Emerging Paradigm in Stroke (STEPS) meeting was organized to bring together clinical and basic researchers with industry and regulatory representatives to assess the critical issues in the field and to create a framework to guide future investigations. (*Stroke*. 2009;40:000-000.)

Key Words: cell therapy ■ restorative therapy ■ stem cells

The field of stem cell biology and regenerative medicine is beginning to generate therapeutic agents for a variety of human diseases and injuries. Stroke is an extremely enticing target for stem, progenitor, or engineered cell therapies because of an urgent need to find therapies that protect at-risk brain cells as well as to promote functional recovery after the initial ischemic event. Multiple possible therapeutic pathways exist for cell therapy treatments of ischemic brain injury. Introducing stem/progenitor and engineered cells into a patient with stroke may lead to integration into areas of cell loss for attempted circuitry repair and functional restoration. Alternatively, the cells may release factors that support the induction of brain remodeling and survival of at-risk cells. It is the latter that appears to have growing support.¹⁻⁵ However, many questions and unresolved issues remain. The Stem Cell Therapies as an Emerging Paradigm in Stroke (STEPS) meeting sought to bring together leading investigators from basic science and the clinical realm along with industry and regulatory representatives to explore these issues in hopes of increasing our understanding of the current status of the field and creating a roadmap for future investigations to accelerate progress toward an effective cellular therapy for stroke. This is particularly relevant as cell therapy guidelines for treatment of stroke are being proposed by investigators and reviewed by

the US Food and Drug Administration (FDA). The STEPS participants focused first on the features of preclinical studies critical to demonstrating the safety and efficacy of cellular therapy for stroke. The group then turned their attention to translation of preclinical studies and optimal design of human clinical trials.

Design Considerations for Preclinical Studies of Cellular Therapy

Several key areas are relevant to the development of cell-based, biological, and pharmacological restorative therapies for the treatment of stroke (Table 1). The appropriate species, type of stroke models, outcome measures and treatment protocols, imaging of cell tracking and host response, the requirement for safety indices, and investigation of mechanisms of action are all important elements of preclinical testing that should be addressed to most effectively and accurately translate research into clinical practice. Preclinical studies must be considered a first step, and differences between animal models of stroke and human stroke may limit translation to clinical trials.

The species of choice for testing restorative therapies is the rat. Studies should test multiple strains and include both adult and aged male and female rodents. Testing in multiple

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Table 1. Considerations for Animal and Human Testing of Cellular Therapy

Mechanism of action
Trophic factors
Cell replacement
Local environment support
Other
Pathological substrate
Ischemic versus hemorrhagic
Peri-infarct versus intra-infarct
Location of pathology
Cortical versus subcortical
Brainstem
Timing after stroke
Acute
Subacute Chronic

laboratories is recommended as well as validation in a second species (ie, mouse). Larger animals, preferably in a nonhuman primate, are desirable, but given the scarcity of an established nonhuman primate stroke model, safety rather than efficacy may be the more appropriate outcome measure.

Stroke models for cellular therapy should focus on focal ischemia, including cortical and cortical/basal ganglia stroke models. The focal brain region damaged by stroke 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 used” is critical in producing the stroke. The resulting pathophysiological manifestations of each stroke model should mimic the human disease condition as closely as possible. Although the results to be garnered are highly relevant to ischemic stroke, the findings can be used as a guide for designing similar preclinical studies for hemorrhagic stroke.

Functional outcome measures should include behavioral tests selected to identify persistent deficits and recovery. The rationale for choosing the appropriate behavioral tests is to align the behavior targeted by the measure to the chosen ischemic stroke model. There is a compelling need to use a rigorous behavioral testing paradigm, including, but not limited to, motor, somatosensory, and cognitive performance. If stroke neuroanatomical damage extends to learning and memory brain structures, the relevant testing should also be pursued. In parallel with producing pathophysiological manifestations of clinical stroke, behavioral correlates in animals should closely approximate the human disease condition. In addition, long-term behavioral testing is recommended and should be performed for a minimum of 1 month after administration of restorative therapies. Standardized behavioral testing regimes should be required for publication and for evaluation of preclinical data by the FDA.

The experimental design for restorative therapies should include: (1) a cell dose–response study to reveal not only the optimal therapeutic dose, but also the maximum tolerable dose; and (2) a cell route of administration study that should be tailored (eg, intracerebral stereotactic delivery or systemic

injections) to the chosen cell-based therapy and its intended clinical use. In addition, a therapeutic window poststroke should be determined as a function of therapeutic dose. This would include the feasibility of repeat dosing regimens to optimize recovery benefit. Cell sources differ, thereby requiring a customized treatment protocol for each cell type. Despite the unique properties of each cell type, the chosen cell population should be characterized *in vitro* using a well-defined set of phenotypic markers that will allow reproducibility across laboratories. The eventual cell deposition and fate (survival, migration, differentiation) of these cells in the brain and other organs after transplantation should be studied both to help define the mechanism of action and to establish safety. In addition to vehicle control, proper cell control groups such as inactivated cell products should be incorporated in the design of cell therapy trials to further delineate transplant effects.

Noninvasive imaging modalities such as MRI may be used to monitor the delivery and tracking of cells and improve understanding of the host response to restorative therapies. Imaging strategies will provide valuable insights into cellular processes that accompany restorative therapies, which to date remain elusive. *In vivo* imaging of the transplant recipients will allow determination of graft- and host-mediated repair mechanisms.^{6,7}

Equally important in advancing restorative therapies is the incorporation of safety outcome measures in the experimental design. Cell therapy research studies should include measures of detecting tumor or ectopic tissue formation, overt behavioral abnormalities, and adverse physiological alterations according to FDA guidelines. Although not a requirement for determination of efficacy and safety, investigation of mechanism of action underlying restorative therapies is critical to the advancement of the field. Cellular mechanisms mediating the therapeutic effects of restorative therapies such as neurogenesis, angiogenesis, synaptogenesis, immunomodulation, cell replacement, and trophic factor mechanisms⁸ should be investigated.

Design Considerations for Clinical Trials

Translation of the knowledge and understanding gained from *in vitro* and animal models to human stroke trials requires attention to the differences between animal and human stroke. The design of human stroke trials using cellular therapy should consider several factors to bridge this gap and optimize the probability of a valid and successful translation. The route of delivery, devices for cell delivery, optimal dose of cells, and stroke subtype are important issues that should be addressed to effectively translate information obtained in animal models to clinical trials.

The choice of delivery method will depend on the type of cell, location of stroke, the patient’s comorbidities and prognosis, and intended strategy to promote recovery (Table 2). The advantage of direct, stereotactic, intracerebral injection is reliable delivery of cells to the damaged area of the brain. The site of injection should correspond to the presumed mechanism by which the cells achieve clinical benefit. Cells that act through a trophic effect might work most effectively in peri-infarct areas, whereas cells designed with replacement

Table 2. Guidance on Cell Delivery Approaches

1. Establish compatibility of cells with delivery device and determine optimal cell density and delivery volume necessary for efficacy
2. Intracerebroventricular: requires further safety and feasibility study
3. Direct intracranial injection: may be most suitable for neural stem cells
4. Intra-arterial: requires demonstration that cells do not lead to microembolism and brain infarcts
5. IV: cells may need homing signal to brain; demonstration that cells do not cause organ toxicity or interfere with organ physiology

and formation of new connections might work best when injected within the infarct. Two completed clinical studies injected cells into chronic basal ganglia strokes with some short-term issues but no long-term adverse events.^{9–11} Cortical, superficial infarcts as opposed to deep subcortical infarcts may be preferable in Phase I and II studies to minimize surgical complications. Certain cell types display significant migratory capacity within the brain and therefore might be ideal for injection in superficial or relatively silent areas even remote from the stroke. Intracerebroventricular injections represent another intracranial approach that could spread cells throughout the brain; however, more animal work is needed to establish its feasibility and safety. A significant concern is that injected cells could adhere to the ventricular wall and cause obstructive hydrocephalus. It also has not been well established that cells delivered by an intracerebroventricular route would migrate through the ventricular surface to the damaged brain.

Animal studies support the selective targeting of cells in the infarcted brain by intracarotid injection of cells.¹² Endovascular drug delivery is routinely performed by interventionalists and is potentially more comfortable for patients than an intracranial route. However, a potential danger of this approach is that certain cell types might adhere to each other and form microemboli. Each cell type should be examined for catheter compatibility and maximal cell density for delivery, factors that may limit the delivery dose. Intracarotid or middle cerebral injections require patency of the proximal arteries. Cells are less likely to reach the area of infarct if they must traverse collateral pathways to reach the area of injury. In some cases, cells may enhance recovery through peripheral mechanisms even without direct entry into the brain.¹³

Intravenous (IV) administration represents the least invasive method of delivery and in many studies has been shown to reliably target cells to the injured brain.¹⁴ Certain cell types such as umbilical cord cells may exert greater benefit when injected IV compared with direct brain injections.¹⁵ To be effective through the IV approach, cells may need a homing signal to travel to the injured areas of the brain. Studies suggest that homing may be mediated by release of chemotactic factors that draw cells to the site of injury.¹⁶ However, injected cells also migrate to perivascular locations in other organs,¹⁴ and it is therefore important to establish that IV delivery does not lead to ectopic growth or elaboration of secreted proteins in other organs. Multiorgan toxicity studies should be assessed in the preclinical package.

The route of administration might differ depending on the time interval from stroke onset. Cell therapy treatment

Table 3. Guidance on Devices to Assist in Cell Delivery

1. Establish cell compatibility with device and establish maximal cell density and delivery volume
2. Establish animal and human compatibility
3. Biodegradability for implanted devices with long-term safety evaluation

initiated early after stroke onset may exert an effect on cell preservation and survival whereas at later time intervals, trophic or replacement effects might help restore lost function. Intravascular delivery and delivery of neurotrophic factors will likely be most effective if given within the first week of stroke, although there is preclinical evidence that the therapeutic time window for IV marrow stromal cells can extend out to 1 month.¹⁷ Intravenous cell therapies that are dependent on chemoattractant signals such as SDF-1 expressed in injured brain^{17–19} and neurotrophic factors should be most effective during active brain angiogenesis and remodeling. Within this early time period, there may be an earlier intervention group at 6 to 24 hours, including intra-arterial-delivered cells and early IV delivery of cells that may achieve and bridge a neuroprotective as well as a restorative effect. A later intravenous cell therapy or neurotrophic factor group at 24 hours to 1 week may target the remodeling processes.

Devices will be increasingly used in the delivery of cells to the brain (Table 3). Both cannulas and microcatheters are already under active investigation in cell therapy trials. Cell adherence and maximal concentration will be important to examine with any device. Volume of delivery will depend on cell density, traffic through the needle, catheter size, size of the vascular tree, and shear stress. Coated stents may be developed that permit release of trophic factors or cell-secreting factors. Surgical implants such as scaffolds and matrices are under development to facilitate cell delivery, migration, and engraftment and to enhance cell persistence for sustained trophic effects. Important parameters to examine include cell compatibility, patient compatibility, induced inflammation, and biodegradability.

Dose-ranging efforts in preclinical models primarily target safety in both acute and long-term studies (Table 4). When clinical trials are initiated, a weight-based translation should be followed based on the optimal dose in animal studies. Brain weight might be used for intracerebral implantations. Dose regimen will be an important factor in optimizing cell therapy and may vary with time of delivery postinjury. Requirements may differ during the early inflammatory phase of stroke compared with postacute injury, particularly if the efficacy of cell therapy is mediated through trophic pathways. A further concern for repeat dosing is immune sensitization.

Table 4. Guidance on Dosing

1. Determine MTD from the literature
2. Determine dose–response curve
3. Initial clinical trials should be based on animal studies of the optimal dose
4. Dose ranges will likely be negotiated with FDA and historical MTD

MTD indicates maximum tolerated dose.

Although much promise has been shown for allogeneic use of adult stem cells based on low immunogenicity and active immunomodulation, little human clinical experience has been collected using such cell types in serial dose regimens. Clinical safety end points for acute infusional toxicity linked to immune sensitization should be included in early trials.

It is possible that some cell therapies for stroke will be regulated as a combination product comprising the active cell therapeutic with a delivery device. Combination products pose an additional regulatory complication. Medical devices are reviewed in an accelerated regulatory pathway compared with cell therapeutics. Device and delivery technology may evolve during the clinical development process, and a regulatory strategy should be developed between the FDA and the scientific community to integrate these advances. It may be prudent to test multiple routes of delivery for a given cell type to provide flexibility in delivery options during clinical testing.

The heterogeneity of stroke raises the issue of which subtypes of stroke to include or exclude in a trial. Because the pathophysiology and mechanisms of recovery differ, ischemic and hemorrhagic strokes should be studied in separate trials. In early-phase studies focused on safety and feasibility, all ischemic stroke subtypes (eg, lacunes, middle cerebral artery territory) and a broad range of stroke severity should be included with care taken to exclude very mild and very severe strokes (eg, including National Institutes of Health Stroke Scale 4 to 22; <4 if deficit is regarded as disabling). In acute-phase studies with an intravascular route, there should be less restriction on location and deficit severity with only the requirement that an infarct is present on MRI–diffusion-weighted imaging/CT. In later Phase IIb trials examining “activity,” and in more delayed trials with intraparenchymal/stereotactic approaches, inclusion criteria should be more restrictive to include more homogenous patients with specific deficits such as focal arm weakness. In Phase II/III trials, methodological quality should be maintained by attention to methods of randomization, blinding of outcome evaluations, and standard clinical trial methodologies for assessing outcomes.

Safety Issues With Phase I Trials

Design of Phase I clinical trials of cell and neurotrophic therapies should be done in close consultation with the FDA Center for Biologics Evaluation and Research. The importance of pre-Investigational New Drug Application discussions with the FDA cannot be overstated. With intravascular delivery of cells, there are concerns of acute infusional toxicities. These are related to cells being filtered and clumping in the lungs, liver, and spleen. Trials of IV delivery of cells should monitor for acute infusional toxicity with daily testing of renal and hepatic function and monitoring in a telemetry setting for 24 hours with pulse oximetry testing and consideration of other pulmonary function testing. Both autologous and allogeneic cells will be used in clinical trials. There are advantages and disadvantages to both. With an intravascular allogeneic approach, appropriate immunological testing to detect and monitor for cell tolerance should be incorporated.

The long-term concern with cell therapy is primarily the risk of tumor formation. The magnitude of this concern is related to the type of cell used. With intraparenchymal injections, only the brain must be monitored for tumor formation, but with IV administration and distribution of cells throughout the body, more extensive monitoring for tumors in other organs will be needed. Appropriate clinical evaluation to screen for systemic tumor before enrollment is essential. Patients with a history of malignancy other than nonmelanoma skin cancers in the past 5 years should be excluded from cell therapy trials. Although an exhaustive malignancy prescreening is impractical, at least a baseline chest x-ray should be required. To monitor for development of brain tumors, follow-up MRI of the brain with gadolinium and/or positron emission tomography/CT imaging for an appropriate interval should be included in the trial design. Registries of patients enrolled in early clinical trials of cellular therapy for stroke should be created for long-term follow-up. Obtaining pathology and autopsy specimens, when available, will be important to examine engraftment and possible tumor formation.

Outcome Measures

Determining the optimal primary outcome measures is challenging in restorative stroke trials. In cardiac trials, there are easily quantifiable measurements such as left ventricular ejection fraction and left ventricular end diastolic volume that serve as primary outcomes.^{20–22} Composite clinical outcome scales such as the modified Rankin Scale and the Barthel Index have been used in neuroprotective trials, but these scales are limited by their lack of specificity for the behavioral targets of the interventions. Detection of significant recovery of language in an aphasic patient or recovery of fine motor control in a patient with hand weakness is best achieved with modality-specific outcome measures.²³ One of the advantages of restorative trials is the availability of a baseline deficit that can be defined in nature, degree, and stability. This will be easiest to establish in studies enrolling patients 3 to 6 months after stroke when most spontaneous recovery has stopped. Trials should include at least 2 pretreatment examinations to assure a stable baseline. Attempts should be made to identify as homogenous a group of patients as possible for cellular therapy stroke trials. There is also an opportunity for repeated-measures analysis in individual patients that can be modality-specific. Phase II trials can test these modality-specific outcome measures as potential primary end points in a larger Phase III clinical trial. It is possible that some restorative therapies will ultimately receive a modality-specific FDA labeling.²³

Exposure of animals with neurological injury and stroke to a stimulating and enriched environment improves functional recovery.^{24–28} Similarly, experience-dependent therapies may improve functional outcomes in patients with stroke.²⁹ The quality and amount of rehabilitation care that a patient receives after stroke can be a potent modulator of functional change in a clinical trial. In a cellular therapy trial, rehabilitation therapy will be a necessary component of efficacy studies. Therefore, every effort should be made to maintain experimental control of the nature and dose of rehabilitation

care. Cell and neurotrophic therapies can be viewed as adjunctive to experience-dependent rehabilitation therapies.

Although functional outcomes should be the primary outcomes in restorative trials, imaging techniques may serve as surrogate measures and provide proof of concept in early-phase trials. Diffusion tensor imaging with tractography permits measurement of the integrity and recovery of white matter tracts,^{30–33} often a deficiency in preclinical studies. Moreover, functional MRI allows the monitoring of effects of these therapies on cortical plasticity and may be used to select patients most likely to respond.³⁴ Tagging and tracking of stem cells with superparamagnetic iron oxide-labeled cells could be pursued to determine the fate of intravascular-delivered cells and their migration in the brain and other organs.^{35–37}

Conclusions

Cellular therapy remains in the early stages of development but holds promise as a means to restore function after stroke. The STEPS conference explored the current status of cell therapy for stroke and the outstanding questions yet to be addressed. Additional information is needed from animal studies to determine effective cell types, optimal timing and route of administration of cell therapy. Many other concerns regarding type of stroke, location, and mechanism require further early-stage clinical trials. To address these issues, animal and human studies must proceed in tandem with a frequent interchange between those involved in both areas. Meetings such as STEPS that integrate free debate between translational investigators, industry, the FDA and the National Institutes of Health allow this interchange to occur in a way that hopefully will lead to improved clinical trial designs based on the best available information and focus investigators on important issues that are most likely to move the field forward. The recommendations generated from this meeting should help guide future laboratory and clinical investigations, increasing the yield and reducing the time to successful implementation of cellular therapies or neurogenic/synaptic factor therapies as effective treatments for stroke.

Appendix

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Disclosures

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References

1. Caplan AI. Adult mesenchymal stem cells for tissue engineering versus regenerative medicine. *J Cell Physiol.* 2007;213:341–347.
2. Chen J, Chopp M. Neurorestorative treatment of stroke: cell and pharmacological approaches. *NeuroRx.* 2006;3:466–473.
3. Tang Y, Yasuhara T, Hara K, Matsukawa N, Maki M, Yu G, Xu L, Hess DC, Borlongan CV. Transplantation of bone marrow-derived stem cells: a promising therapy for stroke. *Cell Transplant.* 2007;16:159–169.
4. Vendrame M, Gemma C, de Mesquita D, Collier L, Bickford PC, Sanberg CD, Sanberg PR, Pennypacker KR, Willing AE. Anti-inflammatory effects of human cord blood cells in a rat model of stroke. *Stem Cells Dev.* 2005;14:595–604.
5. Chopp M, Li Y. Treatment of neural injury with marrow stromal cells. *Lancet Neurol.* 2002;1:92–100.
6. Chopp M, Zhang ZG, Jiang Q. Neurogenesis, angiogenesis, and MRI indices of functional recovery from stroke. *Stroke.* 2007;38:827–831.
7. Guzman R, Uchida N, Bliss TM, He D, Christopherson KK, Stellwagen D, Capela A, Greve J, Malenka RC, Moseley ME, Palmer TD, Steinberg GK. Long-term monitoring of transplanted human neural stem cells in developmental and pathological contexts with MRI. *Proc Natl Acad Sci U S A.* 2007;104:10211–10216.
8. Hara K, Matsukawa N, Yasuhara T, Xu L, Yu G, Maki M, Kawase T, Hess DC, Kim SU, Borlongan CV. Transplantation of post-mitotic human neuroteratocarcinoma-overexpressing nurr1 cells provides therapeutic benefits in experimental stroke: In vitro evidence of expedited neuronal differentiation and GDNF secretion. *J Neurosci Res.* 2007;85:1240–1251.
9. Kondziolka D, Steinberg GK, Wechsler L, Meltzer CC, Elder E, Gebel J, Decesare S, Jovin T, Zafonte R, Lebowitz J, Flickinger JC, Tong D, Marks MP, Jamieson C, Luu D, Bell-Stephens T, Teraoka J. Neurotransplantation for patients with subcortical motor stroke: a phase 2 randomized trial. *J Neurosurg.* 2005;103:38–45.
10. Kondziolka D, Wechsler L, Goldstein S, Meltzer C, Thulborn KR, Gebel J, Jannetta P, DeCesare S, Elder EM, McGrogan M, Reitman MA, Bynum L. Transplantation of cultured human neuronal cells for patients with stroke. *Neurology.* 2000;55:565–569.
11. Savitz SI, Dinsmore J, Wu J, Henderson GV, Stieg P, Caplan LR. Neurotransplantation of fetal porcine cells in patients with basal ganglia infarcts: a preliminary safety and feasibility study. *Cerebrovasc Dis.* 2005;20:101–107.
12. Li Y, Chen J, Wang L, Lu M, Chopp M. Treatment of stroke in rat with intracarotid administration of marrow stromal cells. *Neurology.* 2001;56:1666–1672.
13. Borlongan CV, Hadman M, Sanberg CD, Sanberg PR. Central nervous system entry of peripherally injected umbilical cord blood cells is not required for neuroprotection in stroke. *Stroke.* 2004;35:2385–2389.
14. Chen J, Li Y, Wang L, Zhang Z, Lu D, Lu M, Chopp M. Therapeutic benefit of intravenous administration of bone marrow stromal cells after cerebral ischemia in rats. *Stroke.* 2001;32:1005–1011.
15. Willing AE, Lixian J, Milliken M, Poulos S, Zigova T, Song S, Hart C, Sanchez-Ramos J, Sanberg PR. Intravenous versus intrastriatal cord blood administration in a rodent model of stroke. *J Neurosci Res.* 2003;73:296–307.
16. Chopp M, Li Y. Transplantation of bone marrow stromal cells for treatment of central nervous system diseases. *Adv Exp Med Biol.* 2006;585:49–64.
17. Shen LH, Li Y, Chen J, Zacharek A, Gao Q, Kapke A, Lu M, Raginski K, Vanguri P, Smith A, Chopp M. Therapeutic benefit of bone marrow stromal cells administered 1 month after stroke. *J Cereb Blood Flow Metab.* 2007;27:6–13.
18. Hill WD, Hess DC, Martin-Studdard A, Carothers JJ, Zheng J, Hale D, Maeda M, Fagan SC, Carroll JE, Conway SJ. Sdf-1 (cxcl12) is upregulated in the ischemic penumbra following stroke: Association with bone marrow cell homing to injury. *J Neuropathol Exp Neurol.* 2004;63:84–96.
19. Robin AM, Zhang ZG, Wang L, Zhang RL, Katakowski M, Zhang L, Wang Y, Zhang C, Chopp M. Stromal cell-derived factor 1alpha mediates neural progenitor cell motility after focal cerebral ischemia. *J Cereb Blood Flow Metab.* 2006;26:125–134.
20. Lunde K, Solheim S, Aakhus S, Arnesen H, Abdelnoor M, Egeland T, Endresen K, Ilebakk A, Mangschau A, Fjeld JG, Smith HJ, Taraldsrud E, Groggaard HK, Bjornerheim R, Brekke M, Muller C, Hopp E, Ragnarsson A, Brinchmann JE, Forfang K. Intracoronary injection of mononuclear bone marrow cells in acute myocardial infarction. *N Engl J Med.* 2006;355:1199–1209.

21. Schachinger V, Erbs S, Elsasser A, Haberbosch W, Hambrecht R, Holschermann H, Yu J, Corti R, Mathey DG, Hamm CW, Suselbeck T, Assmus B, Tonn T, Dimmeler S, Zeiher AM. Intracoronary bone marrow-derived progenitor cells in acute myocardial infarction. *N Engl J Med*. 2006;355:1210–1221.
22. Assmus B, Honold J, Schachinger V, Britten MB, Fischer-Rasokat U, Lehmann R, Teupe C, Pistorius K, Martin H, Abolmaali ND, Tonn T, Dimmeler S, Zeiher AM. Transcatheter transplantation of progenitor cells after myocardial infarction. *N Engl J Med*. 2006;355:1222–1232.
23. Cramer SC, Koroshetz WJ, Finklestein SP. The case for modality-specific outcome measures in clinical trials of stroke recovery-promoting agents. *Stroke*. 2007;38:1393–1395.
24. Hicks AU, Hewlett K, Windle V, Chernenko G, Ploughman M, Jolkkonen J, Weiss S, Corbett D. Enriched environment enhances transplanted subventricular zone stem cell migration and functional recovery after stroke. *Neuroscience*. 2007;146:31–40.
25. Dahlqvist P, Ronnback A, Bergstrom SA, Soderstrom I, Olsson T. Environmental enrichment reverses learning impairment in the Morris water maze after focal cerebral ischemia in rats. *Eur J Neurosci*. 2004;19:2288–2298.
26. Nygren J, Wieloch T. Enriched environment enhances recovery of motor function after focal ischemia in mice, and downregulates the transcription factor NGFI-a. *J Cereb Blood Flow Metab*. 2005;25:1625–1633.
27. Komitova M, Mattsson B, Johansson BB, Eriksson PS. Enriched environment increases neural stem/progenitor cell proliferation and neurogenesis in the subventricular zone of stroke-lesioned adult rats. *Stroke*. 2005;36:1278–1282.
28. Dobrossy MD, Dunnett SB. Optimising plasticity: environmental and training associated factors in transplant-mediated brain repair. *Rev Neurosci*. 2005;16:1–21.
29. Wolf SL, Winstein CJ, Miller JP, Taub E, Uswatte G, Morris D, Giuliani C, Light KE, Nichols-Larsen D. Effect of constraint-induced movement therapy on upper extremity function 3 to 9 months after stroke: the EXCITE randomized clinical trial. *JAMA*. 2006;296:2095–2104.
30. Jiang Q, Zhang ZG, Ding GL, Silver B, Zhang L, Meng H, Lu M, Pourabdillah-Nejed DS, Wang L, Savant-Bhonsale S, Li L, Bagher-Ebadian H, Hu J, Arbab AS, Vanguri P, Ewing JR, Ledbetter KA, Chopp M. MRI detects white matter reorganization after neural progenitor cell treatment of stroke. *Neuroimage*. 2006;32:1080–1089.
31. Lindberg PG, Skejo PH, Rounis E, Nagy Z, Schmitz C, Wernegren H, Bring A, Engardt M, Forssberg H, Borg J. Wallerian degeneration of the corticofugal tracts in chronic stroke: a pilot study relating diffusion tensor imaging, transcranial magnetic stimulation, and hand function. *Neuro-rehabil Neural Repair*. 2007;21:551–560.
32. Stinear CM, Barber PA, Smale PR, Coxon JP, Fleming MK, Byblow WD. Functional potential in chronic stroke patients depends on corticospinal tract integrity. *Brain*. 2007;130:170–180.
33. Lai C, Zhang SZ, Liu HM, Zhou YB, Zhang YY, Zhang QW, Han GC. White matter tractography by diffusion tensor imaging plays an important role in prognosis estimation of acute lacunar infarctions. *Br J Radiol*. 2007;80:782–789.
34. Cramer SC, Parrish TB, Levy RM, Stebbins GT, Ruland SD, Lowry DW, Trouard TP, Squire SW, Weinand ME, Savage CR, Wilkinson SB, Juranek J, Leu SY, Himes DM. Predicting functional gains in a stroke trial. *Stroke*. 2007;38:2108–2114.
35. Chemaly ER, Yoneyama R, Frangioni JV, Hajjar RJ. Tracking stem cells in the cardiovascular system. *Trends Cardiovasc Med*. 2005;15:297–302.
36. Neri M, Maderna C, Cavazzin C, Deidda-Vigoriti V, Politi LS, Scotti G, Marzola P, Sbarbati A, Vescovi AL, Gritti A. Efficient in vitro labeling of human neural precursor cells with superparamagnetic iron oxide particles: relevance for in vivo cell tracking. *Stem Cells*. 2008;26:505–516.
37. Zhang ZG, Jiang Q, Zhang R, Zhang L, Wang L, Arniago P, Ho KL, Chopp M. Magnetic resonance imaging and neurosphere therapy of stroke in rat. *Ann Neurol*. 2003;53:259–263.



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