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Stem Cells as an Emerging Paradigm in Stroke 3 Enhancing the Development of Clinical Trials

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Cell-based therapy continues to grow as a new field to explore investigational treatments for stroke. Leaders from academia and industry convened an inaugural meeting in 2007 with members of the National Institutes of Health and Food and Drug Administration (FDA) to generate consensus-based guidelines on the development of cell therapies for stroke, entitled "Stem Cells as an Emerging Paradigm in Stroke" (STEPS).[1] These guidelines focused on preclinical studies that are considered important as part of a development program to support clinical testing of cell therapies. The STEPS meeting also provided recommendations on the conduct of early-stage clinical trials. Given the rapid advances in the field, a second meeting was held in 2009 to update and expand these guidelines, which were published as STEPS 2.[2] In December 2011, investigators in academia, industry leaders, and members of the National Institutes of Health and FDA gathered at a third meeting, STEPS 3, to discuss emerging data on the mechanisms of action of cell therapy, the barriers to successful translation from animal models to patients, and the design of current clinical trials for acute and chronic stroke. Since the prior STEPS meeting, there are now several active cell therapy platforms for stroke and other neurological disorders, in stages that range from preclinical to clinical trials, and with sponsors that include industry, the National Institutes of Health, and the California Institute of Regenerative Medicine. As the field continues to progress and as pilot clinical studies are starting to show safety for some cell types, it has become necessary to formulate a new set of guidelines that address topics not covered in prior STEPS

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*A list of the members of the STEPS 3 Consortium is given in the Appendix.

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publications. Specifically, the current document reflects a compilation of recommendations that focus on more advanced stages of clinical testing, as well as the testing of cell therapies in a broader stroke population that includes chronic stroke.

New Developments in the Field

Cell-based therapies for stroke began as a field during the 1990s, in parallel with studies of tissue transplantation to treat neurodegenerative disorders. Initial pilot stroke studies examined the safety of intracranial delivery of neural cells in patients with stable, chronic basal ganglia infarcts. [3,4] Since the publication of those original reports, hundreds of studies in animal stroke models for more than a decade have shown that various types of cells including those from non-neural sources, such as bone marrow and umbilical cord, can enhance recovery. Rather than cell replacement, the mechanisms of action principally involve stimulation of endogenous repair processes, promotion of brain plasticity and synaptic reorganization, immunomodulation, and reduction in secondary injury. [5,6] Some types of cell therapies, when administered systemically, may not even enter the central nervous system in substantial quantities and instead may indirectly promote stroke recovery by acting on peripheral organs.[7]

Phase I and II clinical studies in stroke are underway around the world, mainly testing safety, with a few trials also exploring signals of efficacy. The majority of clinical trials involve patients in the subacute stages of stroke, although a handful of studies are testing cell-based therapies in patients with chronic stroke. Both autologous approaches, for example, using a patient's own bone marrow,[8,9] and allogeneic approaches, many of which are manufactured by industry, are under investigation. Nearly all cell therapy platforms in registered clinical trials are using non-neural cells. Even before their completion, these trials have raised important questions that were addressed in workshops during the STEPS 3 meeting. The workshops focused on phase II/III trials of cell therapy (Table 1) and establishing guidelines for chronic stroke (Table 2). These workshops generated 6 groups of recommendations:

Phase II/III Trials: Patient Selection

In an early-stage clinical trial in which the primary aim is to assess safety, excluding patients with certain comorbidities (such as renal or hepatic disease) might be helpful to minimize the frequency of adverse events that are more reflective of a patient's chronic diseases rather than effects of the therapy under study. However, use of such an approach in later-stage trials can slow recruitment rates. Use of restrictive inclusion/exclusion criteria is a trade-off between conducting a cleaner and a faster study. A major question is how to balance these competing goals and assure the broadest range of approved usage. One key consideration is to structure entry criteria with respect to properties of the cell therapy of interest, for example, patients with renal dysfunction or a history of neoplasia might be excluded when this is a particular concern for the therapy being evaluated. We favor identifying adverse interactions in preclinical studies, which echoes recommendations for the development of acute stroke therapies.[10] Another key issue is to select patients with respect to the natural history of the stroke syndrome because the null hypothesis in a cell therapy trial is that there

is no difference compared with natural recovery. Most patients exhibit a limited spontaneous recovery, but a subset of patients with stroke remain severely impaired.[11] The expected recovery level of a given patient with stroke may not be predicted with certainty at the time when an acute or subacute cell therapy trial is initiated. Potential exclusionary criteria might introduce a bias in the natural history of stroke recovery by selecting for a patient population with an overall distinct level of recovery. This discussion illustrates that a greater understanding of the natural history of stroke recovery is urgently needed, not just for the design of phase II/III cell therapy studies, but also to inform trials of all therapeutic modalities that have the goal of enhancing stroke recovery.

Study power is reduced when enrolling patients who have a particularly low chance of responding to the cell therapy under study.[12] Several methods show promise for their ability to pro-spectively distinguish responders from nonresponders, [13] for example, by assessing the integrity of key white matter tracts, the functional state of the brain, neurophysiological status, and possibly genetic features. Such investigations might be considered as entry/exclusion criteria, as evidence for their use accrues. At the same time, determining whether such studies can be completed within the optimal therapeutic time window for a particular cell therapy will also be important. Alternatively, selecting patients with no predicted natural recovery may enhance the power of the study to detect an effect. Stratified randomization may allow testing of cell therapies across a range of predicted natural responses based on key physiological parameters.

Patient selection is also intimately connected with study end points. As the focus of clinical trials advances from safety to efficacy, study hypotheses will focus on demonstrating significant behavioral and functional gains. Patient enrollment must be appropriate for the end points used to assess these hypotheses. For example, it will be necessary to enroll patients who have deficits in the domains that will be evaluated as end points—a study focused on improving arm motor function needs to ensure that enrollees have arm motor deficits in the target range at baseline. This issue is also connected with the need to align outcome measures in clinical trials with those used in preclinical studies.

Phase II/III: Time Window

The choice of the time window for patient selection should be informed by preclinical studies. Early-phase safety studies on cell therapies might recruit patients in the subacute to chronic window, where patients have stable neurological deficits and the targeted cell therapy–mediated mechanisms of action might be of secondary importance. However, later-phase efficacy studies should maximally align to the extent possible the treatment time window with the intended pathophysiological targets defined from preclinical supporting data. This issue is complicated by the fact that patients with more severe strokes can deteriorate in the acute stage,[8] making it difficult to separate adverse effects of treatment from the natural history of severe strokes. On the contrary, patients with milder strokes or who have few medical complications are often discharged to an acute rehabilitation facility or home early after stroke onset to reduce length of stay and hospital costs, making it difficult to treat and monitor study patients in a standardized environment.

Trial End Points

The pre-eminent issue for outcome measures is the need to ensure that the end points satisfy regulatory concerns. Global end points have been the main focus of stroke trials to date, for example, in the approval of tissue plasminogen activator. These global end points are based on assessments of disability, rather than impairment, such as the modified Rankin Scale, the National Institutes of Health Stroke Scale, or the Barthell Index. However, evaluation of restorative therapies such as stem cells would benefit from the use of domain-specific end points, which are sensitive to the differences in recovery across different neural systems[14] and more directly relate to the behavioral dysfunction of the affected brain system. These end points include specific measures of motor function such as the Fugyl Meyer, Action Arm Research Test, and walking speed, which have been validated for assessment of stroke outcomes and used in recent large-scale clinical trials.[15–18] The FDA has indicated that domain-specific end points are acceptable as primary outcome measures as long as global end points are retained as secondary outcomes and furthermore that the proposed domain-specific end point needs to be validated. The safety profile of a therapy might also influence the degree to which an end point is deemed to be acceptable. Of course, the priorities for selecting trial end points vary across phases of study, for example, safety versus efficacy. Choosing selective end points is desirable in phase II to detect a signal of efficacy at such a vulnerable phase of development. Indeed, the choice of end points can become a deciding factor in the decision to continue or terminate a cell therapy platform. The issue of end point selection is intertwined with selecting study entry criteria because the cohort enrolled must be appropriate for demonstrating an effect of the cell therapy under study.

Biomarkers of Activity

As was stressed in the prior STEPS 2 publication, [2] there is a great need for markers to gauge the biological activity of a cell therapy. Phase II trials would likely benefit from the inclusion of mechanistic end points. The selection of the route of administration, type of concomitant rehabilitation, and outcome measures might depend, at least in part, on the intended mechanism(s) of action of the cell therapy. At the present time, however, there are no validated biological markers of stroke recovery, although there is ongoing work suggesting potential substrates on neuroimaging that correlate with or even predict good outcomes.[19] These substrates include white matter integrity using diffusion tensor imaging, laterality index using functional MRI, resting state connectivity, motor-evoked potential using transcranial magnetic stimulation, metabolites using magnetic resonance spectroscopy, and structural volumetric assessments of both gray and white matter. A biomarker stroke recovery consortium would help to address this void in the field.

Concomitant Rehabilitation Therapy

A body of research indicates that restorative therapies are maximally effective at improving behavioral outcomes if introduced in parallel with behavioral reinforcement such as rehabilitation therapy.[20] There is evidence in animals with the use of enriched environments or specific behavioral paradigms of limb use that rehabilitation might be a confounding factor in assessing the efficacy of a neural therapy because this enrichment will directly impact the recovery process independently from the therapy and also in possible synergy

with the therapy.[21–23] Furthermore, there is evidence that rehabilitation of patients with stroke has a dose effect, such that the more rehabilitative activity is received, the greater the outcome.[24] Therefore, as a clinical trial advances to phase II, discussing what form of concomitant rehabilitation the patients will receive becomes an important consideration. The importance of rehabilitation will also depend on the intended mechanism of action of a cell therapy. At a minimum, we think that rehabilitation is a variable that must be addressed by the time a cell therapy moves to phase II and will need to be incorporated and specified in detail in any phase III trial. Because patients often pursue rehabilitation therapy outside of study procedures, [25] measuring the total rehabilitation therapy exposure during clinical trial participation may be important.

Several key questions remain: which type of and how much rehabilitation? There is limited consensus regarding standard of care for rehabilitation. What modalities will be delivered, how, and when? As the content of rehabilitation differs across patients, expectations and possibly end points can also differ. Specifying the type of rehabilitation also affects patient selection. Appropriate lessons can be gleaned from trials such as the Locomotor Experience Applied Post-Stroke (LEAPS), [17] in which the patterns of neurorehabilitative activity were prespecified across treatment arms. Specifying rehabilitation content also has implications for the design of late-phase studies, the regulatory pathways, and ultimately labeling. Does a particular cell therapy require rehabilitation to maximize effect or perhaps does the combination lead to synergy? If a cell therapy plus rehabilitation is found to be superior to rehabilitation alone or the agent alone, such a result might lead to labeling as a combination therapy. This notion underscores the value of consulting with regulatory agencies about the role and importance of rehabilitation in any phase III trial, and we recommend specifically for the FDA, End of Phase 2 meeting.

Including some form of rehabilitation into a stem cell intervention, even at the phase II, might improve patient recruitment and interest in a trial in which one arm receives placebo and both arms receive rehabilitation. However, making rehabilitation a requirement for trial participation can be a source of enrollment bias. To what extent might enrolling motivated patients influence end points? No matter the time window, the influence of rehabilitation needs to be taken into account because early rehabilitation within the first few days after stroke has already been shown to be safe[26] and the efficacy of early mobilization is now being studied in the A Very Early Rehabilitation Trial for Stroke (AVERT).

In earlier stages of testing, some studies are simply using a questionnaire to capture the quantity and quality of rehabilitation that patients are receiving. These questionnaires need to be validated to define the type, quantity, content, and duration of rehabilitation therapy a patient undergoes after a stroke.

Chronic Stroke

The majority of ongoing cell therapy clinical trials involving a systemic delivery route are focusing on the acute to subacute stages of stroke given the greater volume of preclinical data in this time period. The mechanisms of action of several types of cell therapies involve stimulation of active repair mechanisms that become operational in the acute to subacute time frame. In addition, some cell therapies modulate the postischemic inflammatory

response in the first few days after stroke. Much less is known about whether cell therapies may improve outcome in chronic stroke. There are a few types of cell therapies in which results from multiple publications have demonstrated improved outcomes when administered at 1 month after stroke,[27–30] a time period in rodents in which stroke enters into a more chronic phase. Few published reports exist on the effects of other cell types in preclinical studies at time points of a month or later, which is noteworthy considering that the first FDA-approved clinical trial of cell therapy for stroke was based on a chronic stroke model.[31] This issue may partly be explained by the lack of preclinical behavioral tests that are sensitive to detect deficits at later time points and the expense of long-term animal maintenance costs.

In contrast to the acute–subacute studies, all prior and existing clinical trials that involve stereotactic intraparenchymal cerebral injection of cell therapies are enrolling patients ≥6 months after stroke onset.[3,4] Some of these trials have unpublished preclinical studies testing cell therapies at later time points. However, the major rationale for treating ≥6 months after stroke is to study patients who have already plateaued in their recovery despite maximal physical and occupational therapy. Under this circumstance, any clinical change might be attributed to the cell therapy, although even in chronic stroke, some patients can achieve gains with structured physical or occupational therapies. Any cell therapy trial must be cognizant of the other therapeutic/ rehabilitative activities of enrolled patients.

At the present time, preclinical models of chronic stroke are limited. There is an insufficient understanding of the science of chronic stroke to provide definitive guidelines regarding efficacy and appropriate patient selection for cell therapy in chronic stroke. We offer the following points and recommendations for future development and testing of cell therapy in this setting.

Recommendations for Future Stem Cell Research in Chronic Stroke

1. Cell therapies that work in acute or subacute stroke may not be efficacious in chronic stroke. Conversely, cell therapies that work in chronic stroke might not be efficacious in acute or subacute stroke.
2. A definition for chronic stroke in humans is needed. We propose to define a chronic stroke as ≥6 months after onset, with no change in deficits for ≥2 months.
3. Testing of a cell therapy for chronic stroke should first be studied in animal models (≥1 month after stroke). Animals should have stable deficits by this time point that can be measured and quantified to test a purported new therapy. A battery of behavioral tests should be included in the characterization of the model and for the purposes of post-translational clinical outcome. Variation of chronicity among different animal models should be considered. As recommended in STEPS 1 and 2, [1,2] multiple models should be investigated in different laboratories. Aged animals, both sexes, and animals with comorbidities should also be considered. Dosing should be consistent with future human applications and scaled appropriately. Primates and other large animal models

are optional, may not be cost effective, and best reserved for questions that cannot be adequately answered in rodent models.

4. Any development program using a cell therapy for chronic stroke should explore and define mechanisms of action in animal models. Any relevant mechanism should be incorporated to the extent possible as an appropriate measure in the design of a clinical trial.

5. For clinical testing, we must be aware of any relevant comorbidities for all possible patients, including pre-existing neurological disorders and cancers. The recovery process will be different if, for example, patients with previous strokes or dementias are included in clinical trials.

6. Use of imaging in clinical trials is strongly encouraged to provide as much information as possible to assess vascular/structural lesions, infarct size, cell viability, location, the success and safety of implantation, and inflammation. Imaging should also be used to monitor safety and recovery and, when possible, to investigate mechanisms of action and provide information on surrogate markers of treatment effect. Imaging measures might also be useful to help stratify patients at baseline.

7. The safest and most effective route of cell delivery should be defined using preclinical studies.

8. Adjuvant therapies: The content of rehabilitation should be evidence based, standardized insofar as possible, and monitored. If enrolled in a cell therapy trial, participation in other types of investigational therapies should be discouraged. We expect that multimodal approaches will be tested in the future, but clinical trials should take into account the additional confounding variables and consider multiarm studies.

9. For any end point in a clinical trial, a clinical baseline before treatment and assessment of stability is important. We recommend domain-specific end points assessing the recovery of sensory, motor, visual, and cognitive functions using validated measures.

10. Biomarkers when available should be incorporated in any clinical trial. Obtaining blood or tissue is important to monitor rejection for any allogeneic study and track any biochemical or imaging data indicative of restorative events.

11. Safety parameters should be followed in some capacity for ≥1 year, with precise study duration for specific stem cell therapies based on preclinical data and discussions with regulatory agencies. Data Safety Monitoring Boards are essential for tracking serious adverse events. We recommend engaging regulatory agencies during all appropriate time points of the clinical trial.

12. Safety trials may be undertaken with all patients receiving active treatment. Early efficacy trials of cell therapies in chronic stroke face the need to establish a plausible biological response with a minimum number of subjects within a short time frame. Assessing treatment effects by change over time in individual

patients with fixed deficits and well-defined natural history without the use of placebo controls may have use in early phase 2 studies in which a clear go/no-go decision is needed. However, a true treatment effect can only be attributed to the study intervention where appropriate control groups are included. Although sham surgeries for stereotactic delivery are invasive, there are reports of patients with chronic stroke reporting immediate improvement after intracranial injection of neural cells.[4] The inclusion of sham controls in later-phase efficacy trials is optimal to distinguish potential effects attributed to placebo, surgery, or the cell therapy, but the risks of sham procedures in the target clinical population must be balanced against scientific desirability. In addition, lack of patient acceptability of sham procedures may compromise recruitment[32] and itself introduce bias. Alternative methods to address the issues raised by sham surgical controls such as crossover trials that permit control patients to receive the study intervention at a later time point or a stepped wedge design in which the study intervention is rolled out sequentially to all participants during succeeding periods of time should be considered as alternatives.

13. The application of neural cells to replace lost brain tissue and reestablish lost connections after a stroke is an important goal but will require extensive advances in basic science before implementation in clinical studies.[33] A range of bioengineering devices including scaffolds and hydrogels are being developed and studied for the purposes of remodeling the brain after stroke and other injuries.

Appendix

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Table 1
Suggestions for Phase II/III Efficacy Trials

1	Structure entry criteria with respect to properties of the cell therapy of interest, the natural history of the stroke syndrome, and study end points to assess hypotheses.
2	The choice of the time window for patient selection should be based on the basis of preclinical studies. Later-phase efficacy studies should maximally align the treatment time window with the intended pathophysiological targets.
3	Evaluation of restorative therapies such as stem cells would benefit from the use of domain-specific end points, which are sensitive to the differences in recovery across different neural systems. These end points should be chosen in phase II to detect a signal of efficacy at such a vulnerable phase of development.
4	At a minimum, rehabilitation is a variable that must be addressed by the time a cell therapy moves to phase II and will need to be incorporated and specified in detail in any phase III trial.

Table 2
Suggestions for Testing Cell Therapies in Chronic Stroke

1	Testing of a cell therapy for chronic stroke should first be studied in animal models (≥1 mo after stroke).
2	Any development program using a cell therapy for chronic stroke should investigate and define mechanisms of action in animal models.
3	Comorbidities need to be taken into consideration when planning a clinical trial, and preclinical studies should be performed, if possible, to evaluate the effects of cell therapy on stroke with comorbidities.
4	Imaging in clinical trials is strongly encouraged to provide as much information as possible to assess safety, vascular/structural lesions, infarct size, cell viability, location, and inflammation, and intended mechanisms of action when possible.
5	The safest and most effective route of cell delivery should be defined using preclinical studies.
6	The content of rehabilitation should be standardized insofar as possible and monitored.
7	We recommend domain-specific end points assessing the recovery of sensory, motor, visual, and cognitive functions using validated measures.