One, Two, Three Steps Toward Cell Therapy for Stroke

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Many clinical trials have failed, despite positive laboratory findings. Stroke clinical trials are no exception, with tissue-type plasminogen activator still the only effective drug for stroke with limited therapeutic window. To enhance the successful outcome of novel therapies in the clinic, initiatives for translational research guidelines have been pursued. In particular, the advancement of stem cell therapy for stroke from the laboratory to the clinic has now been guided by a set of recommendations called Stem Cell Therapeutics as an Emerging Paradigm for Stroke (STEPS). We review here the major criteria for preclinical studies of stem cells arising from the 3 STEPS meetings in an effort to emphasize the need for careful and rigorous assessment of the safety, efficacy, and mechanism of action associated with stem cell therapy for stroke further. Learning from our previous mistakes and identifying gaps in knowledge will likely prevent stem cell therapy from becoming yet another statistic of failed clinical trial in stroke.

Defining the Need for Translational Guidance on Cell Therapy in Stroke

Stroke remains a significant unmet clinical need. Despite knowledge of its pathology, treatment currently is limited to only 1 Food and Drug Administration–approved drug, tissue-type plasminogen activator, with a therapeutic window of only ≤4.5 hours after stroke onset.[1–5] This therapy is effective for few stroke victims because of this narrow window and with serious adverse effects (ie, hemorrhagic bleeding) when tissue-type plasminogen activator is administered beyond this time frame.[5–7] As a result, stroke remains a major cause of disability and death, imposing the need for novel treatments. In laboratory studies, stem cell therapy has proven to be a potential method of regenerating the injured brain beyond the acute phase of stroke.[2,8–11] Laboratory studies and limited clinical trials have shown stem cell transplantation to be a safe and effective therapy for stroke.[12–15] These cells are postulated to assist in cell replacement and secrete factors, which assist in the proliferation and survival of remaining, at-risk cells.[1–5,8–11,16,17] In view of many positive laboratory studies subsequently failing in the clinic, coupled with increasing amount of research involving stem cell therapy, the need for guidelines on standardizing laboratory and clinical procedures has been considered as a translational approach to enhance the successful outcome of cell therapy for stroke in the clinic. The STEPS brought together leaders in stem cell research, industry, and regulatory agencies to create these standards and to provide direction for future research.[9] This article explores the essentials of the preclinical standards and potential research areas stipulated in STEPS.

Overview of STEPS

In 1999, the first Stroke Therapy Academic Industry Roundtable (STAIR) was held to advance drug and device development for the treatment of stroke and to formulate guidelines for the research process, including translation of neuroprotective drugs to clinical trials.[18,19] STAIR became the impetus for STEPS. Recognizing the need for guidelines and direction in stem cell therapy for the treatment of stroke, many authorities on stem cell research, including academics, industry leaders, National Institutes of Health representatives, and Food and Drug Administration representatives, gathered in Washington, DC, in October 2007 to formulate research guidelines after the format of the STAIR meetings.[9,20] The STEPS I proceedings were published in *Stroke* at the end of 2008.[9] Because of the rapid advancement of the field, STEPS II and III meetings, like STAIR meetings, were subsequently held in 2010 and 2011 to update and expand the established guidelines.[18,21] A summary of key recommendations from these 3 STEPS meetings is presented in the Table.

STEPS I

The first STEPS meeting established general guidelines and direction for stem cell research to enhance translation of preclinical studies into clinical trials. STEPS claimed that stroke models should focus on focal ischemia. Rats are the species of choice for preclinical trials to determine safety, functional recovery, optimal timing, dosage, and route of delivery. Nonhuman primate models are desirable to study white matter injury, which is not well characterized in the rat model.[9,22] Studies should test multiple strains of both adult and aged male and female rodents in the preclinical phase. In addition,

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Table. Summary of STEPS Recommendation

Major Translational Guidelines	STEPS Meeting
Rats are species of choice. Test multiple strains of aged and adult male and female rodents	STEPS I
Use control groups with vehicle and dead cells to better determine treatment effects	STEPS I
Perform long-term behavioral tests for at least one month after therapy. Select tests to identify deficits and recovery	STEPS I
Include cell dose response studies to determine optimal dose, delivery device, cell density, and delivery volume	STEPS I
Cells must be characterized in vitro with phenotypic markers to tailor treatment	STEPS I
Test for tumor or ectopic tissue formation, behavioral abnormalities, and physiological alterations that suggest safety concerns	STEPS I
Testing in multiple laboratories is encouraged	STEPS I
Use stroke models with deficits ≤4 wk after stroke	STEPS II
For controls, use vehicle or functionally irrelevant cells and anything correlating with intended clinical protocol	STEPS II
Behavioral testing should occur multiple times for ≥1 mo after the treatment and all outcomes should be reported	STEPS II
Establish a dose–response curve and determine maximum tolerated dose, optimized dose, and minimum dose for benefit in ischemic and hemorrhagic stroke	STEPS II
Publish transcriptional profiles of cells	STEPS II
Cells exhibiting high proliferation or differentiation require long-term safety testing	STEPS II
Evaluate cell deposition, fate, host–cell interaction	STEPS II
Preclinical studies should include many behavioral tests, aged and adult animals of both sexes, some with comorbidities	STEPS III
A better control arm is needed—animals receiving only rehabilitation therapy	STEPS III
Chronic stroke therapy testing should occur ≥1 mo after stroke	STEPS III
Introduce restorative therapy with rehabilitation therapy, which may need to be tailored to individual cell types	STEPS III
Accurate biomarkers to reflect cell activity are needed	STEPS III
Mechanism of action and safest delivery route should be defined in animal models	STEPS III

STEPS indicates Stem Cell Therapeutics as an Emerging Paradigm for Stroke.

control groups such as vehicle and inactivated cells should be included to determine treatment effects better.[9] The cells and their repair mechanisms can be observed in vivo with noninvasive imaging.[23,24] The research should also include cell dose–response studies to determine optimal and maximum dose, optimal delivery device, optimal cell density, and delivery volume. Therapeutic window can then be formulated as a function of therapeutic dose. Administration routes should be studied based on the chosen cell-based therapy. Direct intracranial injection (stereotaxic surgery) may be best suited for neural stem cells, and because cell sources and phenotypes differ, protocol must be tailored to each cell type. This requires characterization of cells in vitro via a well-defined set of phenotypic markers that allows for reproduction across laboratories. Behavioral tests should be selected to identify deficits and recovery, and long-term tests should be performed for ≥1 month after administration of stem cell therapy.[9] Finally, STEPS called for the establishment of preclinical stroke consortia consisting of multiple research institutes, coordinating efforts for multiple laboratories testing the same cells in the same stroke models, using the same standardized tests.

Safety outcomes must also be evaluated for novel therapies. Stem cell treatment studies should test for tumor or ectopic tissue formation, exacerbated behavioral abnormalities, and overt physiological alterations after Food and Drug Administration guidelines. Intracerebroventricular delivery methods necessitate further safety and feasibility research. Intra-arterial delivery requires evidence the cells do not cause microembolism and brain infarcts, and intravenous delivery requires evidence the cells do not interfere with organs and may require a homing signal to the brain.[9] Although not required, the cellular mechanisms regulating the therapeutic effects of stem cell treatment should be investigated as well.[25]

STEPS II

In 2010, because of the exponentially growing stem cell field and the entry of novel types of cells used in stroke therapy, STEPS II was held and the proceedings were published in *Stroke* in 2011.[20] Similar to STEPS I, representatives from academia, industry, and the National Institutes of Health convened again to revisit the guidelines established by STEPS and to identify areas requiring further study in the field.[20] STEPS II largely rehashed the guidelines of STEPS I; however, it added extra emphasis on cell routes, dosing, and clinically relevant experimental design. STEPS II asserted that through laboratory experiments, researchers should establish a dose–response curve after determining maximum tolerated dose from literature, determine an optimized dose and treatment schedule, and establish a minimum threshold for treatment benefit. At minimum, a vehicle solution or functionally irrelevant cells should be used as a control, but other controls at the preclinical level may be necessary to correlate with intended clinical protocol. For example, if immunosuppression will be needed in a clinical study, the immunosuppressive agents alone should be tested along with the cellular product and agents together. Of note, whereas STEPS I recommends the need for inactivated cell products as controls, STEPS II recommends dead cells as additional controls. This is a topic of debate because dead cells and their debris may actually exacerbate stroke outcome, whereas inactivated cells (not dead but functionally stunted) may overcome such adverse effects of dead cells by remaining viable although not functionally active. In addition, cell deposition and fate in stroke models should be evaluated to help define the mechanism of action. Host–cell interaction and methods to improve engraftment of cells when beneficial should also be evaluated.[20] Currently, most cells bolster endogenous repair mechanisms instead of direct cell replacement mode

of action.[26] Evaluation of cell fate can assist in discarding irrelevant pathways and improving clinical trial design. Noninvasive imaging proves useful to explore these issues in vivo. Cell labeling for enhanced imaging requires further research on determining any alterations in the labeled cells' phenotype and functionality. Completely clarifying the mechanism of action, while potentially helpful, is not a requirement for proceeding to clinical trials; however, it presents another area of research lacking exploration.[20]

STEPS II also adds emphasis on mechanism-based investigations that may relate to both efficacy and safety readouts, which were initially highlighted in the recommendations presented in STEPS I. It encourages researchers to publish transcriptional profiles for cells and it recommends stroke models that produce deficits ≤4 weeks after the incident. Clarification on the safety testing is also given. Exogenous cells with a lifespan of days to weeks and cells proven safe in patients already may not require long-term testing in animals.[20] On the contrary, cells that exhibit high proliferation and differentiation likely require long-term and extensive testing to monitor the risk of overgrowth or tumor formation.[27] Transplantable donor cells for stroke therapy include fetal tissue-derived cells, cancer-derived human neuroteratorcarcinoma cells (hNT) or neuroteratocarcinoma-derived neuron-like cells (NT2N) cells, embryonic stem cells, neural progenitor cells, genetically modified cells, immortalized cells, adult stem cells (umbilical cord/blood, bone marrow, peripheral blood, placenta, amniotic fluid, Wharton jelly cells, menstrual blood-derived cells, dental pup, and adipose), and induced pluripotent stem cells. Once safety is established by initial testing of these transplantable cells, treatment efficacy should be assessed. Behavioral testing sensitive to the degree of injury, damage sites, and impairment severity should occur multiple times for at least a month after the treatment, and all outcomes should be reported.[20] Again, testing in multiple laboratories is recommended.

STEPS III

Approximately 2 years later, in December 2011, academics, industry leaders, and members of the National Institutes of Health and Food and Drug Administration gathered again for STEPS III, during which they discussed new research and persisting hurdles in the field. Of note, the rehabilitation therapy experts emerged as a new group of stakeholders in STEPS III. Between STEPS III and the previous meeting, several novel cell therapy platforms emerged, and rapid progress forced STEPS III to compile recommendations for advanced stages of clinical testing (not discussed in this article). Their guidelines, published in *Stroke* in late 2013, emphasized the need for stroke animals to mimic the clinical scenario closely (ie, rehabilitation therapy exposure of stroke subjects), and, as they did before, STEPS III identified areas lacking research.[21]

Both laboratory and clinical research suggest that restorative therapies are most effective when introduced with rehabilitation therapy.[28] The type and frequency of rehabilitation still need to be addressed, and rehabilitation may need to be tailored to individual cell therapies. Additional preclinical research of combination therapy closely mimicking clinical studies can explore these claims.[21] To this end, because patients with stroke are routinely subjected to physical therapy, STEPS III emphasized the need for a better control arm—stroke animals receiving only rehabilitation therapy. This control group will more stringently test the efficacy of cell therapy; if stroke animals receiving transplants fare better than those receiving only rehabilitation, the results will likely translate to improved clinical functions in transplanted patients with stroke.

Several areas of stem cell therapy still necessitate further exploration. Validated biomarkers that reflect the activity of cell therapy in stroke recovery are needed. In addition, the body of research on cell therapy focusing on the chronic stage or stroke pales in comparison with that focusing on the acute to subacute stages. STEPS III offers a series of recommendations for further research of chronic stroke. Effective cell therapies in acute or subacute stroke may not be effective in chronic stroke and vice versa. Testing of chronic stroke therapies in animal models should occur ≥1 month after stroke, once the animals have stable and quantifiable deficits. Preclinical studies should include a large number of behavioral tests, aged animals, both sexes, and animals with comorbidities. The mechanism of action and safest route of delivery should be defined in animal models (Figure).[21] Finally, similar to the recommendations proposed in STEPS I and STEPS II urged, different laboratories should test safety and efficacy of cell therapy in multiple models of stroke.[9,20,21]

Conclusions

Regenerative, cell-based therapy is being approached carefully in the laboratory with rigorous clinically relevant translational studies to advance a safe and an effective therapy for stroke. To provide guidance and direction to this burgeoning field, STEPS has issued and revised a series of guidelines and recommended areas for further research as the field evolves. Major areas identified as lacking research include multiple laboratory testing of safety and efficacy of stem cells (STEPS I), optimization of cell dose and delivery routes appropriate for ischemic and hemorrhagic stroke with consideration of comorbidity factors (STEPS II), and the need for rehabilitation therapy as control arm on which to compare stem cell

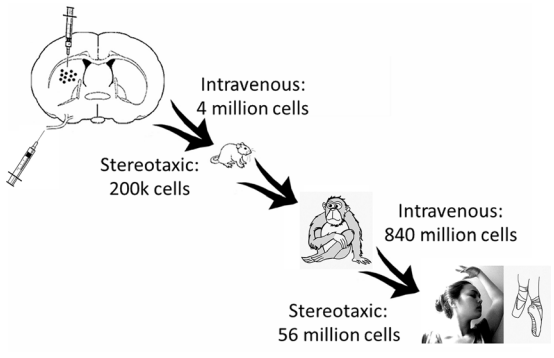

Figure. Both intravenous and stereotaxic intracerebral routes of stem cell delivery are being tested in Food and Drug Administration–approved limited clinical trials for patients with acute and chronic stroke, respectively. Preclinical data that led to these clinical trials were partially collected under Stem Cell Therapeutics as an Emerging Paradigm for Stroke guidelines. These preclinical studies used rat models of stroke, and some cases nonhuman primates to assess cell delivery routes and dosage.

therapy (STEPS III; Table). STEPS primarily targets adult stroke, and extending the guidelines to neonates, presenting with neurodevelopmental problems, such as learning disabilities, and cerebral palsy, has been entertained in Baby STEPS, providing similar translational direction to cell therapy for neonatal hypoxic–ischemic encephalopathy.[29] In addition to Baby STEPS, similar initiatives to enhance the entry of novel therapeutics from the laboratory to the clinic are also being pursued in Parkinson disease, Alzheimer disease, Huntington disease, and epilepsy, highlighting the importance of these translational research guidelines.

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Disclosures

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