A Focused Review of Clinical and Preclinical Studies of Cell-Based Therapies in Stroke

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Stroke occurs in 15 million individuals each year worldwide, and is the second leading cause of mortality.¹ In the United States alone, there are an estimated 795 000 strokes annually, making this the leading cause of long-term disability.² As the population continues to age, the incidence of acute stroke and the prevalence of stroke-related disability are expected to increase. Presently, approximately 7 million Americans are living with chronic disability from stroke.³

Current therapeutic options are primarily focused on the rapid restoration of blood flow during the acute phase of the disease. Significant progress has been made with regard to acute stroke intervention, and endovascular clot retrieval is now recommended as a standard of care in a carefully selected subgroup of stroke patients.^{4,5} However, due to the strict selection criteria for acute endovascular intervention, and the narrow time window during which reperfusion must be achieved, a vast majority of stroke patients do not benefit from acute interventions. Beyond the acute period, physical rehabilitation is the cornerstone of management; however, the benefits of rehabilitative therapy diminish over time, and neurological recovery typically plateaus within 6 months of stroke onset.^{6,7} At the present time, there are no known neurorestorative treatments for chronic stroke.

Cell-based therapy is a promising neurorestorative option for chronic stroke-related neurological disability. In preclinical studies, a variety of cell types derived from humans have been tested in experimental stroke models,^{8,9} including neural stem cells (NSCs)¹⁰⁻¹² and non-NSCs (bone marrow-derived, hematopoietic, umbilical cord, mesenchymal, and multipotent adult progenitor cells).^{9,13-20} In addition to investigating a

range of cell types, preclinical studies have also varied with regard to dose, timing, and route of cell administration, and behavioral outcomes have been variable. Nonetheless, a majority of preclinical studies have demonstrated improvement in some measure of behavioral function, which has justified the translation of cell-based therapies to the clinical setting. Several phase I clinical trials have demonstrated the safety and feasibility of cell-based therapies in stroke patients,²¹⁻²⁴ and early phase II trials have reported mixed results in terms of clinical efficacy.²³⁻²⁶ Similar to the preclinical studies on which these clinical trials are based, there is significant variability in terms of cell type, as well as dose, timing, and route of administration.

In a recently published phase 1/2a clinical trial by Steinberg and colleagues in 2016,²⁷ patients with stable, chronic ischemic stroke underwent intracerebral implantation of carefully specified doses of modified allogeneic bone marrow-derived mesenchymal stem cells (MSCs). This treatment was shown to be safe and well tolerated, and associated with significant improvements in chronic neurological deficits at both 6 months and 1 year. Here, we will review the preclinical and clinical trials that informed the design of this phase 1/2a study.

CELL TYPE

In the 2016 study, Steinberg and colleagues²⁷ utilized SB623, which are modified bone marrow-derived MSCs developed by SanBio, Inc (Mountain View, California) as an allogeneic cell therapy for stroke. These cells are developed by transient transfection with a plasmid vector containing the human *Notch1*-intracellular domain, followed by the administration of certain trophic factors. In a preclinical study, implantation of SB623 cells into the striatum of experimental stroke rats 1 month post-injury resulted in significant improvements in locomotor and neurological function, and significant reductions in peri-infarct cell loss.²⁸

ABBREVIATIONS: Mrs, modified Rankin scale; MSCs, mesenchymal stem cells; NIHSS, National Institutes of Health Stroke Scale; NSC, neural stem cell

A variety of cell types have been used in preclinical and clinical trials of ischemic stroke, and can be broadly classified into neural and non-NSC subgroups. NSCs have the inherent disposition to differentiate into neurons and glia, and thus have the theoretical benefit of replacing damaged neural tissue in the setting of ischemic stroke. In fact, several preclinical studies have demonstrated structural and functional engraftment of transplanted NSCs into ischemic territory,^{11,12,29,30} and NSC transplantation in the setting of experimental stroke has been associated with improved functional recovery.^{12,31} Interestingly however, studies have not demonstrated a consistent association between NSC engraftment/survival and degree of functional recovery.^{32,33} Alternative mechanisms to explain the therapeutic benefit of cell transplantation include increased neural plasticity,³⁴ neovascularization,^{35,36} neuroprotection,³⁷ neurotrophic support,^{13,38} enhanced endogenous neurogenesis,¹⁵ and immunomodulation.^{8,39} These mechanisms provide the rationale for non-NSC transplantation. A wide range of non-NSCs have been studied in experimental stroke, including cells derived from bone marrow, umbilical cord blood, peripheral blood, and mesenchymal tissue. Bone marrow-derived MSCs are multipotent stem cells that are particularly suitable as cell-based therapies due to their ease of isolation (ie, from the bone marrow of autologous or allogeneic donors), immunomodulatory properties (potentially obviating the need for immunosuppression),⁴⁰⁻⁴² ability to migrate to sites of injury,^{43,44} and well-documented safety profile in a variety of clinical contexts.⁴⁵ Additionally, the use of bone marrow-derived MSCs circumvents the ethical scrutiny that complicates other stem cell sources. In a recent meta-analysis of experimental stroke studies, MSC therapy was consistently associated with improvements in multiple measures of behavioral function.⁴⁶

Several prior clinical trials have demonstrated the safety and feasibility of MSC therapy in ischemic stroke. In 2005, Bang and colleagues²³ reported on a phase 1/2 randomized controlled trial of intravenously administered culture-expanded autologous MSCs in patients with subacute middle cerebral artery territory infarcts. This study demonstrated a statistically significant improvement in Barthel index at 3 and 6 (but not at 12) months in the MSC-treated group, as well as a nonsignificant trend towards improved modified Rankin scale (mRS) at all 3 time points. In addition, radiographic atrophy within peri-infarct areas and ex vacuo dilatation of the adjacent ventricle were less prominent in the treatment group. There were no adverse cell-related, serological, or radiographic effects of the MSC therapy.²³ Subsequently, Taguchi and colleagues²⁴ published the results in 2015 of a phase 1/2a nonrandomized clinical trial of intravenously administered autologous bone marrow mononuclear cells in patients with subacute ischemic stroke. Compared with a cohort of historical controls, treated patients demonstrated a statistically significant improvement in mRS, and a trend towards improved NIHSS (National Institutes of Health Stroke Scale) and Barthel index at discharge. Notably, this benefit was more pronounced in the cohort of patients treated with high-dose

cell infusions, as compared with the cohort treated with low-dose infusions.²⁴ In a 2014 randomized controlled trial of intravenously administered autologous bone marrow mononuclear stem cells in 120 patients with subacute stroke, Prasad and colleagues⁴⁷ identified no improvement in any outcome measure, including Barthel index, mRS, NIHSS, or change in infarct volume at 180 days. There were no adverse events associated with this cell therapy.⁴⁷ To date, there have been no comparative studies to suggest the superiority of any particular cell type. Thus, future studies must strive to identify the optimal cell type for treatment of stroke.

CELL DOSE

Steinberg et al (2016)²⁷ divided the enrolled patients into 3 cohorts of 6 patients each, according to the dose of SB623 cells to be administered: 2.5×10^6 , 5.0×10^6 , or 10×10^6 cells in suspension, ranging in concentration from 8000 to 33 000 cells/ μ L.

In a meta-analysis of experimental stroke studies,⁴⁶ MSCs dosages ranged from 3.6×10^4 to 4.3×10^7 MSCs/kg. Interestingly, behavioral scores were inversely correlated with dose ($r = -0.63$, $P = .0003$). Despite this strong statistical correlation, the optimal dose of MSCs for stroke cannot be inferred from this analysis. It remains unclear whether the dose-response curve for MSCs therapy is linear or U-shaped, and the mechanism by which higher MSC doses led to poorer behavioral outcomes in preclinical stroke models also remains to be determined. As mentioned above, a prior clinical study identified a dose-dependent effect of intravenously administered autologous bone marrow mononuclear cells in patients with subacute ischemic stroke;²⁴ however, this was a nonrandomized open-label study of only 12 patients, and the comparison group was a matched cohort of historical controls. Additional studies are necessary to identify the optimal dose of SB623 cells and other cell-based therapies for ischemic stroke.

TIMING OF CELL ADMINISTRATION

The eligibility criteria established by Steinberg et al (2016)²⁷ were designed to identify those patients with stable, stroke-related motor deficit. More specifically, inclusion was limited to chronic stroke patients at least 6 months from stroke onset (mean time poststroke was 22 months), with stable neurological deficits on serial evaluations over a 3-week period prior to enrollment.²⁷ It has been reported that the most substantial improvement in motor function after stroke occurs during the first 30 days, and neurological recovery typically plateaus by approximately 6 months.^{6,7} Thus, any significant improvement in neurological function during the chronic stroke phase is notable and is more likely attributable to the experimental therapy.

Preclinical studies have demonstrated improvements in behavioral outcomes with cell-based therapies administered across a wide range of time windows; however, a meta-analysis of such

studies identified a larger effect size when MSCs were given early (0-8 hours) after stroke onset.⁴⁶ Clinical trials have also varied significantly with regard to the timing of cell administration,⁹ however no head-to-head studies have been conducted thus far to directly compare different therapeutic time windows. It is important to recognize that the underlying mechanism of therapeutic benefit of cell-based treatments may vary depending on the timing of administration: that is, earlier treatment may serve to prevent primary and secondary injury associated with acute ischemia (neuroprotection), whereas later treatments during the subacute or chronic phase of stroke may promote neurorestoration.

ROUTE OF CELL DELIVERY

Steinberg and colleagues²⁷ utilized a magnetic resonance imaging-guided stereotactic technique to define target sites around the periphery of the residual stroke volume. SB623 cells were subsequently implanted intracerebrally along each of three separate tracts approached via a single burr-hole craniostomy using a stereotactic cannula.²⁷

Various routes of cell delivery have been evaluated in both preclinical and clinical studies; these include intravenous, intraarterial, intrathecal, intracisternal, intraventricular, and intracerebral.⁸ Administration of cells directly into the neuroaxis (ie, intracerebral, intraventricular, intracisternal, or intrathecal) offers the theoretical advantage of maximizing the dose of therapeutic cells to the site of injury. There are mixed results reported in the literature as to whether this provides any additional therapeutic benefit over systemic delivery (ie, intravenous or intraarterial).^{48,49} Notably, in a meta-analysis of preclinical studies, all routes of delivery were associated with large effect sizes; however, invasive administration of cell-based therapies directly into the neuroaxis (ie, intracerebral implantation) provided the greatest therapeutic benefit.⁴⁶ Intracerebral and intraventricular implantation also have the inherent disadvantage of requiring invasive delivery procedures, thus making administration logistically more challenging, particularly in the acute stroke period. Clinical studies have also varied with regard to route of delivery;⁵⁰ however, as with other variables related to cell-based therapy for stroke, there is limited comparative clinical data to suggest superiority of 1 particular route of cell delivery over the others. It also remains to be seen whether optimal route of delivery is dependent on additional factors, such as cell type and timing of administration.

DISCUSSION

Due to strict selection criteria and the narrow time window, the vast majority of stroke patients do not benefit from currently available acute stroke interventions. Rehabilitative therapy is primarily aimed at maximizing functional status despite chronic neurological deficits; however, there are no proven neurorestorative treatment options for chronic stroke patients. Cell-based therapies have produced promising results

in preclinical studies of subacute and chronic stroke, and early clinical data suggest this is a safe and feasible treatment option for this patient population. Steinberg and colleagues²⁷ recently published the results of the first clinical trial in North America of intracerebral implantation of modified MSCs in chronic stroke patients, demonstrating safety, feasibility, and efficacy in a small series of patients. In this manuscript, we reviewed the preclinical and clinical trials that informed the design of the clinical trial.

Many unanswered questions remain regarding the optimal cell type, dose, timing, and route of delivery for cell-based therapeutics in stroke. MSCs offer a safe and feasible option that may provide therapeutic benefit via a wide range of mechanisms. Alternatively, NSCs are native to the brain and appear to restore function in preclinical models with similar underlying mechanisms to MSCs. The optimal dose also remains unclear. As described above, there is some *preclinical* evidence of an inverse correlation between dose and behavioral outcome, whereas the limited *clinical* evidence regarding cell dose indicates that higher doses may be beneficial. Steinberg et al (2016)²⁷ utilized 3 separate doses, and there was no identifiable dose dependence of clinical or radiographic outcome, or of safety. With regard to route of delivery, there is no clear clinical evidence to suggest superiority of any particular administration technique. However, several studies have demonstrated relative safety and feasibility of invasive intracerebral delivery of cell-based therapeutics, and this route of administration provides the theoretical benefit of maximizing dose delivery to the target location. The optimal timing of cell delivery is likely to vary depending on the goal of therapy. That is, administration during the acute period may serve to minimize primary and secondary injury from stroke, whereas subacute or chronic delivery of cell-based therapeutics is more likely to provide a neurorestorative benefit. The decision to limit inclusion to chronic stroke patients in the study by Steinberg and colleagues²⁷ was intended to allow each patient to act as their own control, since neurological recovery had plateaued and neurological exam stabilized with little chance of further recovery. Additional studies will be necessary to evaluate the therapeutic effect of SB623 cells in acute and subacute stroke patients as well. Currently a phase 2b multicenter, double-blind control study in 156 patients is underway using intraparenchymal delivery of SB623 cells (2.5×10^6 cells, 5×10^6 cells, or burr hole only). Future trials must also investigate the interaction of each of these variables, identify the optimal combination of cell type, dose, timing, route of delivery, and patient selection for the treatment of stroke.

Cell-based therapy holds great promise for improving the outcome in patients suffering from stroke. However, this treatment is still in the early stages. Many fundamental mechanistic issues still need to be elucidated in preclinical studies. Further phase 1, 2, and 3 studies should be pursued, emphasizing the importance of controls in the later stage trials.

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