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Stem Cell Recipes of Bone Marrow and Fish: Just What the Stroke Doctors Ordered

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Abstract Stem cell therapy for stroke has advanced from the laboratory to the clinic, but remains as an experimental treatment. Two lines of transplant regimens have emerged, namely the "early bird" peripheral injections in subacute stroke patients and the "late night" direct intracerebral treatments in chronic stroke patients. Autologous bone marrow-derived stem cells, which only required minimal manipulations during graft cell preparation, gained fast-track entry into the clinic, while gene modified stem cells necessitated overcoming more stringent regulatory criteria before they were approved for clinical use. Safety of the stem cell therapy can be declared from these clinical trials, but efficacy warrants further investigations. Here, we offer insights into the translation of cell therapy from the laboratory to the clinic, in the hopes that highlighting the lessons we learned from this experience will guide the optimization of functional outcomes of future clinical trials of stem cell therapy for stroke.

Keywords Ischemic stroke · Stem cell therapy · Clinical trials · Translational research

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An Appetizing Lab-to-Clinic Translation of Stem Cell Therapy for Stroke

Among the many novel regenerative medicine strategies tailored towards brain injury repair, stem cell-based therapeutics have thus far proven safe and effective in animal models of stroke. We address here key lab-to-clinic translational research parameters that relate to efficacy, safety, and mechanism of action underlying stem cell therapy, as well as discrepant transplant regimens between preclinical and clinical studies. Recently published reports of clinical trials on stem cell therapy for stroke have advanced two lines of targeted treatment regimens, namely the peripheral stem cell transplantation for subacute stroke patients and the intracerebral transplantation for chronic stroke treatment. Understandably for safety reasons, the early entry of stem cell therapy to the clinic involved autologous transplantation of bone marrow-derived stem cells, as well as minimally invasive delivery routes, using the intra-venous (The STem Cell Application Researches and Trials In NeuroloGy-2 or STARTING-2 Study; NCT01716481 [1]; Safety/Feasibility of Autologous Mononuclear Bone Marrow Cells in Stroke Patients; NCT00859014 [2]; Intravenous Autologous Bone Marrow-derived Stem Cells Therapy for Patients With Acute Ischemic Stroke or InveST; NCT01501773 [3]) and the intra-arterial (Autologous Bone Marrow Stem Cells in Ischemic Stroke; NCT00535197 [4]) in subacute stroke patients. Although preclinical studies of direct intracerebral transplantation have been explored much earlier than the peripheral routes of stem cell transplantation, satisfying the regulatory requirements presented much challenge thereby delaying in translating such invasive application into the clinic, as well as changing the target patient population to chronic stroke patients (Pilot Investigation of Stem Cells in Stroke or PISCES; NCT01151124 [5]; A Study of Modified Stem Cells

in Stable Ischemic Stroke or ACTIsSIMA; NCT01287936 [6]). A careful examination of these clinical trials reveals that some stroke doctors ordered stem cell recipes with bone marrow as main entree, while others preferred an à la carte of fish and marrow garnished with special blends of genetically modified spices. With preliminary results now being reported from on-going clinical trials of stem cell therapy for stroke, a cautious assessment of the accurate functional benefits of this novel treatment will further direct the future of regenerative medicine for neurological disorders.

Stem cell therapy has been examined in numerous neurological disorders, with highly encouraging results suggesting its indication as a stroke treatment [7–9]. By targeting the subacute and chronic phases of stroke, stem cell therapy extends the effective time of intervention, potentially leading to significant benefits in many patients. Aside from factors as cell route, dose, and timing of administration, the specific type of stem cells is key to the successful translational lab-to-clinic outcomes of cell transplantation [10–12]. Several types of transplantable cells have been tested in the laboratory, with a few selected for clinical trials in stroke [13–18]. Because of the adult tissue origin and the resemblance with many of the stem cell phenotypic features, bone marrow-derived stem cells have emerged as leading transplantable cell type for CNS disorders, including stroke [19]. Moreover, primarily due to solid safety profile in other disease indications, especially hematologic diseases, preclinical studies and ongoing clinical trials have chosen stem cell recipes based on bone marrow and its cellular derivatives [6–20]. Among these bone marrow-derived stem cells, mesenchymal stem cells (MSCs) have been extensively studied in stroke animal models. MSCs have been shown to exhibit adult multipotency characteristics [20–27] and to produce functional recovery, including decreased brain damage and improved motor and cognitive performance [8, 19–23, 26, 28] upon transplantation in stroke models. Postulated mechanisms of action mediating the functional effects of cell therapy in stroke involve cell replacement, growth factor secretion, and promotion of endogenous brain repair processes, such as neurogenesis, angiogenesis, synaptogenesis, and recently biobridge formation [29–33].

Clinical trials have been initiated, and preliminary reports have demonstrated safety, although efficacy and mechanisms of action warrant additional investigations [6]. These clinical trials can be categorized into the "early bird special" peripheral (intravenous, intra-arterial, and intranasal) transplants of stem cells, and the "late night special" of direct intracerebral implantation of bone marrow-derived stem cells [3, 6] in subacute stroke and chronic stroke, respectively.

Are the "Early Bird" Peripheral Transplants in Subacute Stroke Patients really Special?

Autologous MSCs gained fast-track entry in a few clinical trials. In an open-labeled study, autologous intravenous bone marrow transplantation, using 7–10 million mononuclear cells (MNCs) per kilogram, delivered subacutely (24 and 72 h after stroke), produced robust clinical improvements in a modified Rankin scale over a 6-month period in many of the transplanted patients with no detectable adverse events [2]. A subsequent phase II, multicenter randomized trial, involving 58 patients transplanted with a mean of 280.75 million MNCs at median of 18.5 days after stroke onset, revealed no improvements in the Barthel index score, modified Rankin scale shift analysis, National Institutes of Health Stroke Scale (NIHSS) score, or infarct volume compared to non-transplanted stroke patients at 6-month post-transplantation [3]. This study indicated safety, but not efficacy of intravenous transplantation of MNCs in subacute stroke. Using a subset of immunoselected CD34+ bone marrow MNCs, 100 million autologous cells were intra-arterial transplanted within 7 days of onset in 5 stroke patients diagnosed with severe anterior circulation ischemic stroke (NIHSS score of ≥8) [4], which resulted in improvements in the modified Rankin scale and NIHSS score, coupled with reductions in lesion volume, and no adverse events during a 6-month follow-up period. A much longer delay following stroke (i.e., 4 weeks after onset) was pursued in another intravenous administration of two booster shots of 50 million MSCs (once at 4 weeks and another at 6 weeks after onset) in 16 stroke patients, who displayed improvements in neurological outcomes using the modified Rankin Scale, with no significant side effects, over a 5-year follow-up period [1].

Fish and Marrow "Late Night" Specials: Not a Matter of Personal Taste

The use of cell therapy for chronic stroke treatment utilized stem cells that were garnished with genetic modifications [5, 6] as recently reviewed [34]. The UK-based ReNeuron Phase 1 trial called PISCES [5] employed CTX-DP neuronal cell line derived from human fetal brain, while the US-based SanBio, Inc. Phase 1/2A trial, called ACTIsSIMA [6] used SB623, bone-marrow derived MSCs.

The "fish" PISCES trial utilized stereotactic putaminal transplantation of CTX-DP cells 6–60 months after ischemic stroke in 11 men aged 60 years or older with stable disability based on NIHSS score ≥ 6 and modified Rankin Scale score of 2–4 [5]. Single intracerebral doses of 2–20 × 10^6 CTX-DP cells produced some improvement of neurological and functional outcomes over 24 months post-transplantation, with no detectable cell-related adverse events [5]. The ACTIsSIMA

trial that involved 18 chronic stroke patients showed that at 12 months post-transplantation some transplanted patients displayed significant improvements from baseline (mean increase of almost 7 in European Stroke Scale, mean decrease of 2 in NIHSS, mean increase of >19 in Fugl-Meyer total score, and mean increase of >11 in Fugl-Meyer motor function total score) accompanied by brain imaging indicating tissue recovery readouts [6]. Both PISCES and ACTIsSIMA trials were based on small number of patients, thereby dampening the interpretations about efficacy of cell therapy in stroke.

Reneuron employed the c-mycER(TAM) technology to achieve conditional growth control with a fusion protein comprising a growth promoting gene, c-myc, and a hormone receptor regulated by the synthetic drug, 4-hydroxy-tamoxifen (4-OHT) in producing CTX-DP immortalized cell line derived from human first trimester fetal cortical cells [35]. SanBio transfected human bone marrow-derived MSCs with a Notch intracellular domain (NICD)-expressing plasmid to generate neuronal-like SB623 cells [36]. Compared to the unmanipulated or minimally manipulated MSCs, both genetic modified CTX-DP and SB623, while representing highly homogenous cell population, took longer time to gain clinical approval partially due to the gene therapy clinical trials that had resulted in the deaths of a number of patients [37]. Accordingly, despite CTX-DP and SB623 passing the homogenous stem cell litmus test, the appetite for gene-modified stem cell product required a more stringent palatable regulatory oversights. Indeed, long-term in vivo stroke animal modeling studies were mandated by UK Regulatory and US FDA to demonstrate the safety and efficacy, as well as the mechanisms of transplanted CTX-DP and SB623 cells [18, 38–40]. A critical safety outcome measure precludes silencing or deleting the gene inserted into respectively CTX-DP and SB623 cells prior transplantation was a major obstacle in getting regulatory approvals. With CTX-DP, silencing of c-mycERTAM transgene was achieved following growth arrest (epidermal growth factor, basic fibroblast growth factor, and 4-OHT withdrawal) in cell culture, while CPG methylation was indicated as the transgene silencing machinery following intracerebral implantation into stroke animals [41]. For SB623, transient NICD transfection prevented the transfected nucleic acid from being expressed in daughter cells [42].

This "two-course meal" (gene-amplified homogenization and gene silencing/restriction) arguably modified the stemness and functional properties of CTX-DP and SB623. In particular, with the lineage commitment of the cells relegated to a neuronal phenotype, the capacity of these neuronal-like cells to migrate is likely reduced. As a consequence, transplant regimen was dictated by the limitations of the final stem cell product instead of addressing the palate of the stroke patient, thereby forcing the trials to ignore the debilitating brain disorder in favor of an invasive intracerebral transplant approach to circumvent the lessened migratory potential of CTX-DP and SB623. Moreover, whereas the intracerebral approach entailed lower effective dose range of transplantable cells compared to systemic transplantation, the initial targeted patient population consisted of severe stroke patients, as is the general case of early phase regulatory (e.g., FDA) approved clinical trials for experimental invasive treatment interventions. Considering that critically ill patients would serve as subjects for these clinical trials, this meant much higher threshold for efficacy outcomes.

Getting Acquainted with the Stem Cell Menu

Altogether the clinical trials of cell therapy in subacute stroke demonstrate that transplantation of MSCs and their cellular derivatives (including MNCs) appears to be safe in stroke, although its efficacy, largely due to small number of patients and the open-labeled approach, is still under debate. The discrepant transplant regimens across the four systemic transplantation protocols prevent vis-à-vis comparisons of outcomes [43]. The cell dose, timing and route of delivery widely differ across trials (Table 1). Likewise, the inconsistency between laboratory and clinical transplant regimens raises the question whether the current clinical protocols retain robust scientific foundation to merit as evidence-based medicine

Table 1 Clinical transplant protocols

Clinical trial	Authors	Cell type	Route	Dose	Timing	Subject size
NCT01716481 (Starting-2)	Lee et al. [1]	Bone marrow – MSC	IV	50×10^6 (2 booster shots)	Subacute - 4 weeks after stroke	85
NCT00859014	Savits et al. [2]	Bone marrow – MNC	IV	100×10^6 cells/kg	Subacute – 24-72 h after stroke	10
NCT01501773 (Invest)	Prasad et al. [3]	Bone marrow – MSC	IV	280.75×10^6	Subacute – 18.5 days after stroke	120
NCT00535197	Banerjee et al. [4]	Bone marrow – HSC	IA	100×10^6	Subacute – <7 days after stroke	82
NCT01151124 (PISCES)	Kalladka et al. [5]	CTX-DP - Neural	IC	$2–20 \times 10^6$	Chronic – 6-60 months after stroke	13
NCT01287936 (ACTIsSIMA)	Steinberg et al. [6]	SB623 - Neural	IC	$2.5–10 \times 10^6$	Chronic – 6-60 months after stroke	18

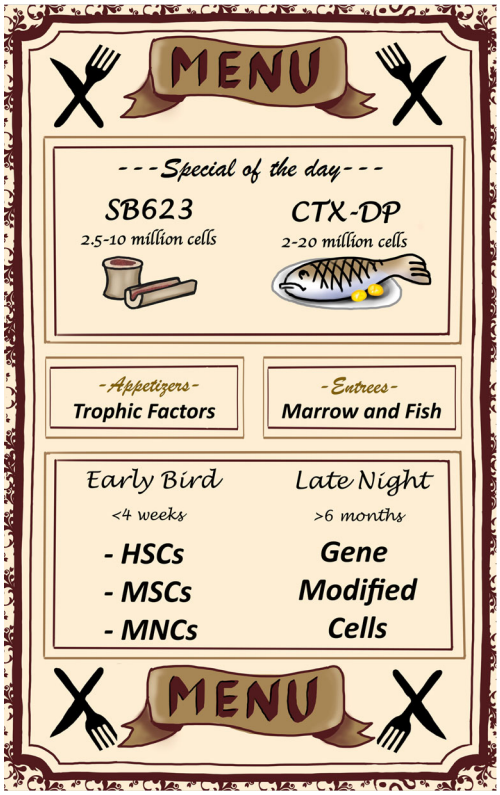

Fig. 1 Cartoon depicting the gastronomic marketplace of stem cells for stroke therapy

[12]. Furthermore, distinct donor cells were utilized in the trials, which perhaps essentially account for the varying clinical results. Based on the donor starting material alone, comparisons of the outcomes from the four trials will be inconclusive. Lab-to-clinic recommendations from the Stem cell Therapeutics as an Emerging Paradigm for Stroke (STEPS) [11] may improve the clinical design and eventually the functional outcomes of cell therapy for stroke.

In digesting the clinical data from the two trials of cell therapy in chronic stroke, that CTX-DP and SB623 cells are genetically modified cells necessitates long-term monitoring of transplanted patients, because despite solid gene silencing and restriction machinery, the cells' non-tumorigenic fate was demonstrated exclusively in experimental stroke animals. The possibility exists that an amplified c-mycERTAM transgene expression or stable transfection of NICD may activate oncogenes leading to tumor or ectopic tissue formation when human CTX-DP or SB623 cells are transplanted in human stroke patients.

As additional clinical trials proceed with enlisting larger cohorts of patients, long-term follow-up, and thorough assessment of the status of the transplanted cells, we will be able to further evaluate the safety, efficacy, and mechanism of action of stem cell therapy for stroke. In this regard, to gain a deeper understanding of the target patient population, the clinical trials on systemic transplantation of MSCs in subacute stroke patients and the CTX-DP and SB623 intracerebral transplants in chronic stroke should also be assessed in a vis-à-vis fashion, coupled with bed-to-bench side preclinical studies to optimize the stem cell transplant regimen in stroke patients. Finally, noting that stem cell therapy for stroke remains an experimental treatment, extreme caution must be exercised when interpreting the limited regulated clinical trials, but more so we should be vigilant against unregulated clinical procedures operating under the guise of medical tourism.

Conclusions

The stroke brain can be repaired [44, 45]. Regenerative medicine via stem cell therapy for stroke has been shown as safe and effective in preclinical studies. MSC and its derivatives, due to their long track record of safety as donor cells, have gained early entry as leading graft source for cell therapy in stroke. The use of immortalized cell lines and genetically engineered cells may also complement MSCs as graft material, but we learned that their translation to the clinic was fraught with much regulatory obstacles. Notwithstanding the solid safety outcomes from these clinical trials, the demonstration of efficacy of cell therapy in stroke remains elusive, owing in part to inconsistent clinical translation of the optimal transplant regimen established in the laboratory. A gastronomic marketplace of stem cells for stroke therapy abounds (Fig. 1), but one needs to approach the banquet with caution before uttering the proverbial phrase, Buon Appetito!

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Compliance with Ethical Standards

Potential Conflicts of Interest CVB receives grant support from SanBio, Inc., Karyopharm, Inc., International Stem Cell Corp., and royalties from Athersys, Inc., and has patents and patent disclosures related to stem cell therapy for stroke.

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