Clinical Trials: Intracerebral Cell Therapy in Stroke Patients

11

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11.1 Introduction

The use of stem cell therapy for stroke is a burgeoning area of clinical research. Buoyed by promising studies performed in preclinical animal models of stroke, in which transplanted cells resulted in functional improvement (Borlongan et al. 1998; Chen et al. 2001a, b; Chopp and Li 2002; Guzman et al. 2008; Hicks et al. 2009; Stroemer et al. 2008), the study of intracerebral cell therapy for stroke has progressed to early phase clinical trials.

Though it is clear that transplanted cells convey a functional benefit, the mechanism by which this occurs is not fully known. Transplanted cells are hypothesized to provide benefit not only directly through cell replacement of damaged tissue but also by providing trophic, neuroprotective, and immunomodulatory support. Lack of clear mechanism is but one of the challenging considerations encountered in the design of clinical trials. Particular concerns that may impact the potential success of cell therapy include the anatomy, vascular supply, timing of stroke, site and technical

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delivery of cell implantation, target patient population, and selection of appropriate outcome measures (Locatelli et al. 2009; Savitz et al. 2004).

A variety of cell types have been investigated in experimental models and translated to clinical trials. Published trials of intracerebral cell therapy studied cells derived from embryonic carcinoma lines (Kondziolka et al. 2000, 2005), fetal porcine striatum (Savitz et al. 2005), and bone marrow (Suárez-Monteagudo et al. 2009). More recently, investigators around the world have continued to evaluate the use of both autologous marrow-derived (NCT00950521) and allogeneic cord blood-derived (NCT01438593) cells, as well as neural stem cells (NCT01151124). Current studies in the United States have focused on the use of modified marrow stromal cells (NCT01287936). This review summarizes the four published clinical trials of intracerebral cell therapy for stroke and describes the ongoing study of such therapy in the United States (Table 11.1).

11.2 Preclinical Basis for Clinical Trials

Several investigators have evaluated the use of transplanted fetal tissue, rat striatum, or cellular implants in small animal stroke models (Johansson and Grabowski 1994; Kleppner et al. 1995). Although transplanting primary human fetal neurons into patients with neurodegenerative disease may exhibit favorable preclinical results, the widespread use of such cells is likely to be limited due to ethical and logistic difficulties inherent in obtaining large quantities of fetal neurons (Thompson et al.

Table 11.1 Overview of published and ongoing clinical trials of intracerebral stem cell therapy for stroke

Reference	Cell type	Study design	Number of patients	Outcome	Trial status
Kondziolka et al. (2000)	NT2	PI, R-SB	12	Safe and feasible	Complete
Kondziolka et al. (2005)	NT2	PII, R-SB	18	Safe and feasible. No significant benefit in motor function	Complete
Savitz et al. (2005)	Fetal porcine LGE	PI, NR	5	Terminated by FDA after AEs in two patients	Complete
Suárez-Monteagudo et al. (2009)	Autologous bone marrow-derived mesenchymal stem cells	PI, NR	5	Safe and feasible	Complete
NCT01287936	Bone marrow stromal cells	PI/IIA, NR	18	–	Ongoing

Abbreviations: *NT2N* NTERA-2 human embryonic carcinoma-derived cell line, *PI* phase I, *PII* phase II, *R* randomized, *SB* single blind, *LGE* lateral ganglion eminence, *FDA* food and drug administration, *AE* adverse event

1999); thus, much effort has been devoted to developing alternate sources of cells for use in transplantation.

The first of such sources to be translated into the clinical realm was the NTERA-2 cl.D1 (NT2) human embryonic carcinoma-derived cell line. These cells were shown to proliferate in culture and further differentiate into pure, postmitotic human neuronal cells (LBS-Neurons) upon treatment with retinoic acid (RetA) (Andrews et al. 1984; Pleasure and Lee 1993). During induction, the LBS-Neuronal precursor cells undergo significant changes resulting in the loss of neuroepithelial markers and the appearance of neuronal markers. The final product is a >95 % pure population of human neuronal cells that are virtually indistinguishable from terminally differentiated, postmitotic neurons (Andrews et al. 1984; Pleasure and Lee 1993); accordingly, they appear to function as central nervous system (CNS) progenitor cells with the capacity to develop mature neuronal phenotypes. When transplanted into mouse models, NT2 cells survived, extended processes, expressed neurotransmitters, formed functional synapses, and integrated into the host tissue (Kleppner et al. 1995; Trojanowski et al. 1997).

Preclinical studies of LBS-Neurons were carried out in rat models of transient focal, rather than global, ischemia to maximize the chance of functional recovery. In several studies, animals received ischemic insults localized to the striatum. Animals that displayed significant behavioral deficits 1-month post-insult received cell transplantation with LBS-Neurons and cyclosporine A (CsA) treatment. Over the 6-month observation period, animals displayed amelioration of ischemia-induced deficits, including complete recovery in the passive avoidance test and normalization of motor function in the elevated body swing test. These benefits contrasted with control groups of rats receiving fetal cerebellar cells, medium alone, or CsA alone, which failed to show behavioral improvement (Borlongan and Sanberg 1995; Borlongan et al. 1997; Saporta et al. 1999). Subsequent studies demonstrated graft survival with mature neuronal phenotypes and integration into the host brain (Kleppner et al. 1995; Trojanowski et al. 1993, 1997). Viable cells were demonstrated in 90 % of recipients, with graft survival observed up to 14 months posttransplant, and electrophysiologically tested differentiation into fully mature neuronal phenotypes (Borlongan and Sanberg 1995; Kleppner et al. 1995).

A second alternate source of cells investigated for neurotransplantation was porcine primordial striatum, also known as the lateral ganglion eminence (LGE). Cells harvested from the LGE are known to develop into striatal GABAergic projection neurons and were first evaluated in animal models of Huntington's disease; graft survival and host integration, as well as improvement of functional neurologic deficit were demonstrated (Deacon et al. 1994; Isacson et al. 1995; Pakzaban et al. 1993). In these early studies, animals followed for up to 15 months were found to have axons and glial fibers with maturational changes typical of pig striatum (Isacson et al. 1995). Studies of immunosuppressed rats with lesions of the corpus striatum analyzed grafts for development with respect to donor age, cell dosage, and survival up to 22 weeks postimplant. Prolonged development of striatal cells was observed, with long-distance, target-specific axonal growth into the host brain (Deacon et al. 1994). Later studies of LGE cells in rat models of middle cerebral artery (MCA)

occlusion showed that cells transplanted to ischemic striatum 3–28 days after stroke led to implant survival, with solid grafts observed to fill the infarct cavity; cells differentiated into glia and neurons, elaborated extensive processes into the host brain, showed evidence of synaptogenesis, produced neurotransmitters, and expressed typical neuronal proteins. Fourteen days post-stroke, these animals showed significant functional improvement compared to controls (Dinsmore et al. 2002).

Cells derived from bone marrow have also been investigated for use in cell therapy for stroke patients. In addition to hematopoietic stem cells, bone marrow contains mesenchymal stromal cells and a portion of multipotent cells that differentiate into tissues of mesenchymal lineage, such as osteoblasts, chondroblasts, adipocytes, and skeletal muscle (Tang et al. 2007). Two studies of CD34+ cells harvested from bone marrow and given either systemically or incracranially to animal models of stroke demonstrated evidence of functional recovery and reduced infarct size (Shyu et al. 2006; Taguchi et al. 2004). Further studies of rat MCA occlusion models have demonstrated improved recovery with mesenchymal cells administered in a variety of manners and beginning 1-day post-infarct. Delayed delivery, even as far as 1-month post-infarct, continued to convey long-term functional improvement (Shen et al. 2007). These studies were translated into successful clinical trials of IV mesenchymal stem cells; treatment in both the acute and subacute post-stroke period was found to be safe and feasible on short- and long-term follow-up (Bang et al. 2005; Honmou et al. 2011; Lee et al. 2010; Savitz et al. 2011b). Intracerebral delivery, however, results in more transplanted cells in the brain directly targeting the lesion when compared to systemic modes of administration (Jin et al. 2005). Though the underlying mechanism remains unclear, given that only a small percentage of cells have been observed to survive for long periods near the ischemic region or express neuronal markers, it is less likely that they exert benefit by replacing cells (Coyne et al. 2006) and perhaps more probable that they enhance functional outcomes by indirectly supporting repair mechanisms (Luo 2011) through trophic factors or specific cell types, such as the CD34+ subpopulation (England et al. 2012).

One recently developed cell line utilizing bone marrow is SB623 (SanBio, Inc., Mountain View, CA), which is a line of human bone marrow-derived stromal cells transiently transfected with a plasmid encoding the intracellular domain of Notch-1 (Dezawa et al. 2004), a human heterodimeric transmembrane receptor important for transcription activation. In vivo rat models of MCA occlusion have been treated with CsA and escalating doses of intracranial SB623 at 1-month post-infarct. As early as 7 days posttransplant, these animals showed significant behavioral improvement on both elevated body swing test and Bederson score, with a trend toward a dose response with escalating numbers of cells (Yasuhara et al. 2009). Interestingly, only 7–9 % cell survival, with less than 1 % neural differentiation, was observed, supporting a trophic mechanism of action. Later studies with 3- and 6-month follow-up showed similar recovery of motor and neurologic function, with continued improvement over time, as well as the ability to transplant cells safely without the use of CsA (Yasuhara et al. 2009).

11.3 Clinical Trial Design

Thus far, only early phase clinical trials with small patient populations have been performed. Trials recruited fairly diverse study populations; however, study participants were all adults >18 years old with "stable" motor deficit (unchanged over a period of time) from "chronic" stroke occurring from 3 months up to a maximum of 10 years prior to baseline. All studies excluded patients with severe or uncontrolled chronic diseases, malignancies, or disabling psychiatric conditions.

LBS-Neurons were the first human cells to be tested in clinical trials. A phase I open-label single-blind study was performed with primary safety and secondary efficacy outcomes. Twelve patients with basal ganglia stroke 6 months to 6 years prior to transplant and stable motor deficit were recruited. Two cohorts were examined—the first comprised of four patients receiving two million cells and the second comprised of eight patients randomized to either two or six million cells. Patients were followed for 52 weeks, and the primary outcomes were analyzed at 24 weeks postoperatively (Kondziolka et al. 2000).

A subsequent phase II open-label single-blind trial was performed. Potential subjects must have been age 18–75 years and experienced an ischemic or hemorrhagic infarction involving the basal ganglia 1–6 years prior to enrollment with no substantial change in neurological deficit for ≥2 months. Two cohorts of nine patients each were randomized to receive either cells plus rehabilitation or rehabilitation alone. In the first cohort, patients received five million cells plus rehabilitation ($n=7$) or rehabilitation alone ($n=2$). In the second cohort, patients received ten million cells and rehabilitation ($n=7$) or rehabilitation alone ($n=2$). Patients were followed at predetermined intervals for 52 weeks, with primary outcome for statistical analyses examined at 6 months. Studies evaluated safety of transplantation using neurologic exams, serial MRI and positron emission tomography (PET), laboratory tests, and documentation of all adverse events. The phase I trial used the National Institutes of Health Stroke Scale (NIHSS), European Stroke Scale (ESS), Barthel Index (BI), and SF-36 Health Survey to evaluate functional disability and quality of life. In addition to these assessments, the phase II trial also included use of the Stroke Impact Scale (SIS), Fugl-Meyer Assessment of Motor Recovery After Stroke, gait tests, Action Research Arm Test, and Grooved Pegboard Tests (Kondziolka et al. 2000, 2005).

Similarly, Savitz et al. (2005) performed an open-label trial with intentions to recruit 12 subjects. Inclusion criteria varied somewhat; however, patients were limited to only those with ischemic stroke from 3 months to 10 years prior to baseline with an MCA infarct of 20–100 cm^3 and affecting the striatum, resulting in permanent neurologic deficits and moderate disability. Patients were followed serially for safety and efficacy up to 24 months. Evaluations included physical examinations, NIHSS, Modified Rankin Scale (mRS), BI, MRI, and laboratory tests, which notably included polymerase chain reaction for porcine endogenous retrovirus.

For autologous bone marrow transplant, a small, open-label trial of five patients was planned. Participants were required to be between 40 and 70 years old with disabling motor sequelae from stroke occurring between 1 and 10 years prior to

enrollment. Investigators studied a comprehensive battery of safety and efficacy outcomes. Neurological status was assessed using NIHSS, Scandinavian Stroke Scale (SSS), BI, and SF-36 in addition to scales evaluating spasticity, gait, and equilibrium. Neurocognitive status was assessed using the Mini-Mental Status Examination (MMSE); Wechsler Adult Intelligence Scale (WAIS); Rey memory, learning, and complex figures tests; and a selection of additional attention, memory, language, frontal executive function, and depression scales. Neurophysiologic and radiologic assessment consisted of electroencephalography (EEG), SPECT, MR spectroscopy, and transcranial magnetic stimulation (TMS). Patients were assessed at baseline, 6 and 12 months postoperatively.

The ongoing trial of SB623 cells (NCT01287936) being conducted at the University of Pittsburgh and Stanford University is an open-label phase I/IIA with primary safety and secondary efficacy outcomes. Eighteen patients between 18 and 75 years of age with ischemic stroke in the MCA territory occurring 6 months to 3 years prior to baseline will be recruited. Participants must have "stable" neurologic deficits of NIHSS > 7 and mRS of 3–4 with no change during the 3 weeks prior to enrollment. Three dose-escalation cohorts of six patients, each receiving 2.5, 5, or 10 million cells, will be assessed. Similar to prior trials, safety outcomes evaluated will include record of adverse events, physical examinations, MRI, and laboratory tests for 24 months postoperatively. Efficacy parameters to be assessed include NIHSS, ESS, mRS, Fugl-Meyer, changes on PET scan, and a comprehensive neurocognitive battery.

11.4 Cell Preparation and Surgical Method

Cell preparation prior to the day of surgery was cell and source specific. All investigators used stereotactic surgical procedures for intracerebral injection of cells with either computed tomography (CT) or magnetic resonance image (MRI) planning.

LBS-Neurons (Layton BioScience, Inc., Gilroy, CA) were produced using antibiotic-free conditions in a class 10,000 clean room according to cGMP protocols. The NT2/D1 human precursor cell line was plated in culture from a well-characterized working cell bank. This stock culture was passaged twice per week in DMEM/F-12 growth media. NT2/D1 cells were then induced to differentiate with 10 μM RetA. After 6 weeks of treatment, cultures were harvested with trypsin/EDTA and replated at lower cell densities, then maintained in DMEM/F12 media containing 5 % FBS and a mitotic inhibitor mixture for 6 days. Cells were selectively harvested, purified, and extensively tested, then cryopreserved in freezing media and stored in the vapor phase of liquid nitrogen. On the day of surgery, 1 h prior to implantation, vials were thawed, gently washed twice with Isolyte S (McGaw, Inc., Irvine, CA) and centrifuged at 200 × g for 7 min at room temperature, then the cell pellet was resuspended in Isolyte S. Viable cell count was determined with a sample of LBS-Neuron suspension using 0.4 % trypan blue, and the cells were resuspended to a concentration of 3.3×10^7 cells/mL in Isolyte S and aliquoted at 120 μL/sterile 1.0 mL vial. An aliquot was considered acceptable if greater than 50 % of cells were

viable and cells were immediately transferred to the operating room (OR) upon completion of preparation (Kondziolka et al. 2005).

A stereotactic surgical procedure was used for intracranial injection of cells. CT was used for operative planning of safe trajectories that entered a cortical gyrus and spared a sulcus. In the phase I trial, the first four patients received a single-pass injection of two million cells divided into three implants of 20 μL each. On a brain weight basis, this was 1/20th of the effective dose in rats. The subsequent eight patients received either a single, two million cell pass or three-pass injections of six million cells in nine implants (Kondziolka et al. 2000). In the phase II trial, five cell implants, spaced equally across a distance of 20–25 mm, along five trajectories, for a total of 25 deposits, were planned. One point in the basal ganglia inferior to the center of the stroke and four other targets anterior, posterior, medial, and lateral to the central target, spaced by 5 mm, were chosen (Kondziolka et al. 2004). A stabilizing probe, 1.8 mm in outer diameter and 15 cm long, was inserted first to a point 4 cm proximal to the final target. Cells were injected at a rate of 5 μL/min, for a total implantation time of approximately 150 min (Kondziolka et al. 2005).

When studying autologous bone marrow neurotransplantation, Suárez-Monteagudo et al. (2009) required patient donation on the day prior to surgery. Patients were taken to the OR, and samples of 120–150 mL of bone marrow were aspirated from the posterior iliac crest, collected in sterile plastic bags containing CDP-adenine, and transported to the cell culture lab for processing. Mononuclear cells were isolated by a Ficoll-Hypaque density gradient and resuspended in heparinized (10,000 UI/L) saline, according to institutional practice (Hernandez et al. 2007). A 40–60 mL concentrated cell suspension was obtained, washed twice with saline, and centrifuged at 1,200 × g for 10 min at 4 °C. DNAse (0.003 %) was then added to the cell suspension and centrifuged at 650 × g for 5 min. The pellet was resuspended in an appropriate volume of saline and stored at 4 °C until transplant 24 h later. A small fraction was used for cell counting, viability testing by trypan blue exclusion, and microbiological tests. CD34+ cell analysis was carried out using fluorescence-activated cell sorting.

Surgical planning was performed using CT images and STASSIS software (CIREN, La Habana, Cuba). The final implant location was based on combined information from single-photon emission tomography (SPECT) to quantify blood flow, 1.5 T MRI for high anatomic resolution, and consideration of accessibility, size, topography, and morphology of the lesion. Recordings of multi-unit electrical activity were performed transoperatively using a deep recording system (NDRS, CIREN, La Habana, Cuba) to confirm existence of neuronal activity in the perilesional area. Cells were implanted using several tracts around the target, using a Rehncrona canulla and eight deposits of 2.5 μL per tract (Suárez-Monteagudo et al. 2009).

For surgical implantation of SB623 cells, 1 mL sterile suspensions containing 5×10^6 cells/mL cryopreserved in CRYOSTORE™ freezing media are provided by SanBio within 2 weeks of the surgical date. On the morning of surgery, the preserved cells are thawed, washed, centrifuged, and resuspended in Plasma-Lyte A at the necessary concentration for administration to the patient within 3 h of resuspension.

After applying a standard stereotactic frame (Leksell Stereotactic System, Elekta), 1.5 T MRI is used for surgical planning, identifying a trajectory that enters a gyrus and spares a sulcus. Targets are identified in the basal ganglia inferior to and above the motor region of the stroke. Five cell deposits, spaced 4–5 mm apart with 2–3 implants within the penumbra distal to the stroke area and 2–3 implants within the penumbra proximal to the stroke area, along three trajectories, for a total of 15 deposits, are delivered. Accessible target locations closest to the motor pathways are selected. After creating one 1–1.5 cm burr hole and opening the dura, a long stabilizing cannula (1.8 mm outer diameter, 15 cm long) with a removable solid stylet is inserted to a point just proximal to the penumbra of the stroke area. Following stylet removal, a Pittsburgh Implantation Cannula (0.9 mm outer diameter, 19 cm long, 20 μL total volume) is inserted to the deepest target point. Cells are injected at a rate of 10 μL/min and 20 μL/deposit, for a total deposit of 100 μL/tract and implantation time of approximately 60 min, with a maximum allowable time of 3 h from cell preparation to final implantation (NCT01287936).

11.5 Need for Immunosuppression

The decision to immunosuppress patients undergoing neurotransplantation may be based on a number of factors, including the source of graft, type of cell product, and consideration of any immunomodulatory effects the cells may have (Savitz et al. 2011a). Patients participating in trials of LBS-Neurons were treated with oral CsA, 6 mg/kg/day for 1 week prior to surgery and 8 weeks postoperatively during the phase I trial (Kondziolka et al. 2000) and 6 mg/kg twice daily 1 week prior to surgery and for 6 months postoperatively in the phase II trial (Kondziolka et al. 2005). Patients also received intraoperative methylprednisolone (40 mg IV) during both studies.

On the other hand, no other clinical trials have required immunosuppression of study participants. Xenografts of LGE cells were treated with anti-MHC class I $F(ab')_2$ fragments lacking the Fc region (PT85, Veterinary Medicine Research and Development, Inc.) prior to transplantation. Graft survival has been shown to persist using this immunosuppressive technique after intracerebral porcine cell transplantation in animal models of Huntington's and Parkinson's disease and stroke (Dinsmore et al. 2002; Pakzaban et al. 1995).

Further, studies of autologous bone marrow transplant would not require immunosuppression given the etiology of cells. Lastly, preclinical studies of rats treated with SB623 cells with and without CsA showed no significant differences in improvement; compared to controls, rats receiving SB623 still exhibited significant benefit, even without treatment with CsA. Additionally, histologic evaluation of experimental and control groups displayed no eventful inflammation or immune response and no obvious difference between the two specimens. Markers of immunoreactivity were comparable across all treatment groups. The lack of differences in inflammatory and immune markers provides evidence that intracerebral transplantation of SB623 does not elicit overt host reactions (Yasuhara et al. 2009).

11.6 Results

11.6.1 Patient Population

Patient demographics and clinical characteristics for each of the completed trials are described in Table 11.2. The phase I trial of LBS-Neurons recruited 12 patients (9 men, 3 females) age 44–74 years old with a mixture of cardiac, renal, endocrine, and psychiatric comorbidities. There were no significant differences between cohorts for age, height, and weight. Mean time since onset of infarct was 27 months (range 7–55), with stroke involvement limited to the basal ganglia in eight patients and including an additional extensive region of cortex in four patients. Comparatively, the phase II trial recruited 18 patients (13 men, 5 women) age 24–70 years old with a similar mixture of comorbidities. Mean time since onset of stroke was 3.5 years (range 1–5); nine were ischemic and nine were hemorrhagic.

Due to adverse events, the study of LGE cells was stopped early after enrollment of five patients. Of those treated, three were men and two were women. Patients were aged 25–52, and mean time since onset of stroke was 5 years (range 1.5–10). All patients had MCA strokes with contralateral hemiparesis, and a mixture of right- and left-sided infarcts was included. Comorbidities were minimal and included hyperlipidemia, diabetes mellitus, partial seizure (one patient), use of a baclofen pump (one patient), and factor V Leiden.

Investigators studying autologous bone marrow transplant recruited a total of five patients, as well; participants were three men and two women, aged 41–64 years old, who were 3–8 years post-stroke. Both ischemic and hemorrhagic strokes were included and were located in the thalamus, striatum, or primary motor cortex.

11.6.2 Cell Viability

Cell viability was only minimally reported in published clinical trials. Kondziolka et al. (2000, 2005) required >50 % viability for administration, and Savitz et al.

Table 11.2 Patient demographic and clinical characteristics

Reference	Mean age, years (range)	Females (F), males (M), count	Type of stroke, count	Mean time since onset, years (range)
Kondziolka et al. (2000)	61 (44–74)	3 F, 9 M	12 ischemic	2.25 (0.58–4.8)
Kondziolka et al. (2005)	59, 5 M 58, 10 M 46 control (24–70)	5 F, 18 M	9 ischemic, 9 hemorrhagic	3.5 (1–5)
Savitz et al. (2005)	40 (25–52)	2 F, 3 M	5 ischemic	5 (1.5–10)
Suárez-Monteagudo et al. (2009)	51 (41–64)	2 F, 3 M	3 ischemic, 2 hemorrhagic	5 (3–8)

(2005) required >70 %; however, individual patient viability data was not reported. This may reflect the controlled, highly predictable, and reproducible nature of laboratory preparation of cells prior to administration. In contrast, studies of autologous bone marrow neurotransplantation utilized participant donation on the day prior to surgery; thus, quality and viability of cell samples were much more unpredictable compared to trials of other cell types. To this end, Suárez-Monteagudo et al. (2009) reported cell implant viability operative data. Preoperatively, injected cell volume ranged from 115 to 220 μL with 76–94 % viability. Total number of injected cells ranged from 14 to 55×10^6 cells; notably, total number of transplanted cells and %CD34+ cells were not directly correlative with injected volume. Postoperatively, viability ranged from 50 to 92 %. Patients received 46–88 deposits of cells distributed in 6–15 tracts.

11.6.3 Safety

Implantation of LBS-Neurons was deemed safe and feasible upon completion of phase I and phase II clinical trials. Implantation was successfully performed in all 26 patients with no evidence of hemorrhage or new neurological deficit identified in the immediate postoperative period. Two new neurological events did occur; however, one patient experienced a single seizure the day after implantation and one patient, who was on aspirin and ticlopidine after surgery, was found to have a chronic subdural hematoma requiring surgical drainage 1 month after surgery. A small risk for both of these events should be expected, given the cortical transgression, spinal fluid loss, and minor accumulation of air in the brain during the procedure that may increase risk of seizure and the frequency with which stroke patients are managed with antiplatelet agents or other anticoagulants conveying some risk of delayed intracranial hemorrhage. In the long term, no adverse events related to implantation occurred at 24–36 months follow-up for phase II patients nor did phase I patients experience any adverse events at 52–60 months after implant (Kondziolka et al. 2005).

Unfortunately, use of LGE cells was associated with complications in some subjects. Though there were no perioperative complications and the first 3 patients did not experience adverse events during the 4 years following implantation, the fourth patient (with history of right MCA infarct) developed progressive left arm and leg weakness at postoperative day 20. MRI showed an area of enhancement in the right frontal lobe remote from the area of infarct and surgical implant site. Subsequent biopsy showed bland necrosis with macrophages and T cells, as well as adjacent areas of organization suggestive of infarct; it was negative for pig repetitive DNA element. The patient returned to baseline after 10 days and a course of steroids. An independent study board concluded that he experienced a cortical vein occlusion, likely secondary to the surgical procedure. The right frontal lobe enhancement resolved after 5 months, and after a complex partial seizure when the patient decreased his gabapentin at 6 months, he had no further adverse outcomes. Patient 5 also experienced an adverse outcome consisting of generalized and partial complex seizures while hyperglycemic (glucose up to 630). MRI showed a ring-enhancing

lesion in the right frontal lobe, subadjacent to the site of the first burr hole, and an area of mild enhancement within the infarct. The ring-enhancing lesion resolved within 3 months and the mild enhancement remained unchanged on follow-up imaging. On long-term follow-up, the patient experienced no further clinical events related to cell implantation. Following the adverse events experienced by patients 4 and 5, the study of LGE cells was terminated by the Food and Drug Administration (FDA) (Savitz et al. 2005).

Patients who received autologous bone marrow cell therapy did not experience any clinically significant adverse events related to the surgical procedure or cell implantation. Investigators classified health events in the 90-day postoperative period as "surely related," "probably related," "less probably related," and "not related." The only "surely related" or "probably related" events were headache, drowsiness, nausea, and hyperglycemia, which all resolved within 24–48 h after the surgery. In the long term (90 days–1 year), no adverse events related to the surgery or cells were observed (Suárez-Monteagudo et al. 2009).

11.6.4 Functional Outcomes

All studies used combinations of validated tools for assessment of neurologic deficit and level of function in stroke patients; number and type of outcome measures were varied, as were time points and length of follow-up for assessment of efficacy of therapy.

Studies of LBS-Neurons used ESS as the primary evaluation of functional improvement, which is validated for changes ±3 points. For the phase I study, baseline mean ESS was 60.1 for the two million cell group and 61.3 for the six million cell group. At the 24-week follow-up, 6 of 12 patients had improved ESS (range 3–10 points), 3 patients were unchanged, and 3 patients deteriorated (range −1 to −3 points). Mean change from baseline to 24 weeks for all patients was 2.9 points ($p = 0.046$), and there was no correlation between clinical outcome and size of infarct or time since infarction. No statistically significant change was seen in NIHSS, BI, or SF-36 at 24 weeks, though a trend toward improvement was observed for NIHSS. At 2 years, the mean ESS change was −1.4 in the two million cell group and +8.5 in the six million cell group ($p = 0.05$).

For the phase II study, ESS was once again used as the primary functional outcome; scores recorded at weeks 24, 26, and 28 were averaged to account for variations in patient effort at any one particular visit. Of the 7 patients receiving 5 million cells, 4 had improved ESS scores (range 5.3–15 points), 2 were unchanged, and 1 was decreased (−4.5 points) compared to baseline. Comparably, in the ten million cell group, 2 of 7 patients had improved scores (6.5 and 14.5 points), 2 were unchanged, and 3 had decreased scores (−4.5 to −5.5 points). Of the controls, 1 of 4 patients had an improved score (3.5 points) and the other 3 remained unchanged. For all patients receiving LBS-Neurons, mean change in ESS from baseline to 6 months was 2.7 points, compared to a mean change of 0.75 points in controls ($p = 0.148$). When comparing mean 6-month ESS scores to mean baseline scores of

treated patients, the observed change of 4.74 points was not significant ($p=0.146$). No difference in NIHSS was observed when comparing all patients who received cells to controls or when comparing the five and ten million cell groups. The SIS, a measure of degree of disability caused by stroke and the effects of surgery, was higher (higher daily activity) in treated patients compared to controls ($p=0.056$), though significant change was only noted when comparing scores for patients with implants at 6 months and baseline ($p=0.045$). Everyday memory scores in patients with implanted cells were also significantly improved compared to controls ($p=0.012$) and when comparing 6-month scores with the patients' own baseline scores ($p=0.004$). Though no significant change in Fugl-Meyer scores was noted when patients receiving LBS-Neurons were compared with controls, a trend toward improvement in hand movement (mean change 1.15 points [95 % CI 0.07–2.4], $p=0.06$) and wrist movement (mean change 0.92 [95 % CI 0.05–1.9], $p=0.06$) was observed. Authors also evaluated grasp, grip, pinch, and gross movement using the Action Research Arm Test. At 6 months, treated patients had significantly improved gross-movement scores compared to controls. Overall score in treated patients compared to their own baseline was significant in both the five million cell group ($p=0.043$) and ten million cell group ($p=0.051$). Significant changes were observed in gross movement and grasp but not grip and pinch movements.

When investigating use of LGE cells, Savitz et al. (2005) used NIHSS, mRS, and BI. Anecdotally, authors reported that several patients experienced improvement in baseline aphasia, motor strength, ambulation, and spasticity; however, only one patient had significant change in NIHSS (>4 points), comprised of speech improvement from aphasia/dysarthria (incomprehensible speech) to fluent speech with only very occasional word finding difficulty, as well as mild improvement in right arm strength. All reported changes were sustained after 4 years of follow-up. No significant changes in functional outcome as measured by mRS or BI were reported nor did any patient experience deterioration compared to baseline.

Using an extensive battery of functional outcome measures, authors investigating autologous bone marrow cell therapy reported statistically significant functional improvement. Compared to baseline, patients showed improved motor neurologic condition based on both NIHSS and SSS at 12 months ($p<0.05$). They also showed reduced spasticity as measured by the Ashworth scale ($p<0.05$) and increased functional capacity assessed using BI ($p<0.05$). Equilibrium and locomotion were also significantly improved as early as 6 months postoperatively. Though no significant change in SF-36 was reported, all five patients did report improvement of the item concerning limitations due to the physical condition (Suárez-Monteagudo et al. 2009).

11.6.5 Neuropsychological Outcomes

Neuropsychological testing was included in the phase II study of LBS-Neurons and in the study of autologous bone marrow transplant. Both studies aimed to assess similar measures of verbal intelligence, mood, and five domains of cognitive

function (language, attention, learning and memory, visuospatial/constructional ability, mental flexibility). Testing was done at baseline and at 6 months following LBS-Neuron implantation and included the New Adult Reading Test, Controlled Word Association Test, subtests from the Wechsler Memory Scale III, Rey Complex Figure Test, Rey Auditory Verbal Learning Test, Block Design from the WAIS, Beck Depression (BDI-2), and Beck Anxiety Inventories (BAI). There was no evidence of depression in any patient, either at baseline or 6 months. Few changes were associated with treatment on any neurocognitive test, with the exception of one; significant improvements were evident on the Rey Complex Figure Test, which assessed visuospatial/constructional ability and nonverbal memory sensitive to lesions in the nondominant hemisphere. The four patients who demonstrated the greatest improvement in this area all had strokes in the nondominant hemisphere; all of these patients employed a more organized approach and demonstrated improved image reproduction on both immediate and delayed recall. Overall, patients who have strokes in the nondominant hemisphere tended to demonstrate greater improvement in visuospatial/constructional skills and in nonverval memory compared to those who had strokes in the dominant hemisphere; for example, in nondominant stroke subjects, the difference in improvement on the Rey Figure Test Immediate Recall exceeded two standard deviations compared to dominant stroke subjects (Stilley et al. 2004).

Authors reported the results of neuropsychological testing in patients receiving autologous bone marrow cell therapy as either improved, no change, or worsened, with differences defined as postoperative change greater than one standard deviation when compared to the patient's own baseline for each measure. Summaries of intellect, attention, verbal memory, nonverbal memory, language function, frontal/executive function, and depression were reported. Results were mixed and difficult to interpret. One patient showed a trend to worsen in several areas, with the exception of noted improvement in nonverbal memory. The remaining four patients showed a trend to improve in most functions; however, no one specific area was more notable than the others (Suárez-Monteagudo et al. 2009).

11.6.6 Imaging

With the exception of the lesions observed following LGE implantation and one chronic subdural hematoma following implantation of LBS-Neurons, described above, no anatomic or structural changes were noted with any cell therapy on follow-up MRI. There was no evidence of edema, contrast enhancement, mass effect, or change in the appearance of the infarct following treatment with LBS-Neurons. Notably, significant differences were seen in [^{18}F] fluorodeoxyglucose (FDG) uptake on PET scans at 6 and 12 months postoperatively. At baseline, all patients in the phase I study of LBS-Neurons showed marked focal hypometabolism corresponding to the MRI-defined stroke territory. Ipsilateral hypometabolism was variably observed, as was cerebellar diaschisis. At 6 months, >10 % increase in relative FDG uptake in the stroke area was observed in 7 of 11 patients; this increase was

sustained at 12 months in 3 of 11 patients. By 12 months, 5 of 11 patients had at least one postimplantation scan demonstrating a >10 % rise in relative glucose metabolism over baseline. Interestingly, one patient did demonstrate paradoxical decrease in thalamic stroke and surrounding area after implantation. When 6- and 12-month data were pooled, postimplantation increase in metabolic activity relative to baseline in the stroke and surrounding regions significantly correlated with results on the motor subscale of the ESS ($p=0.02$ and $p=0.006$, respectively). No difference in patients receiving two or six million cells was noted. Decreases in the magnitude of contralateral cerebellar diaschisis, which was identified in a majority of cases, also correlated with clinical functional improvement in NIHSS and ESS scores ($p=0.009$ and $p=0.02$, respectively) (Meltzer et al. 2001).

Rather than undergoing PET scans, patients receiving autologous bone marrow therapy were followed using EEG, MR spectroscopy, SPECT, and TMS. At baseline, EEG showed epileptic-like activity in 3 of 5 cases and ipsilateral slow activity in all cases. At 6 months and 1 year postoperatively, epileptic-like activity was detected ipsilaterally to the lesion in all five cases, though no clinical seizures were ever observed. MR spectroscopy showed no significant increase in the N-acetyl-aspartate/creatine ratio when compared to baseline at 6 and 12 months. Temporal changes were noted, however, with a decrease in the immediate postoperative period that reached a nadir at 3 months and then recovered by the 1-year follow-up. Functional mapping using TMS and assessment of regional blood flow using SPECT yielded no significant changes on follow-up imaging.

11.7 Discussion

To date, four small early phase clinical trials of cell therapy for patients with motor deficit from stroke lasting >3 months have been reported. The main objective of these early phase studies was to demonstrate safety and feasibility of cell therapy for stroke patients, with secondary goals of establishing efficacy. Results of these studies suggest that allogeneic transplant of cultured neuronal cells and autologous transplant of bone marrow are safe and feasible, both with respect to the surgical procedure and on long-term follow-up (Kondziolka et al. 2000, 2005; Suárez-Monteagudo et al. 2009). Xenografted porcine fetal LGE cells exhibited less favorable results (Savitz et al. 2005).

Establishment of efficacy, assessed using various scales that measure neurologic and functional deficit resulting from stroke, has proved more elusive. Trials of LBS-Neurons demonstrated some improvement in ESS for patients receiving cells. Interestingly, the group of patients receiving five million cells had both a greater number of ischemic strokes and a greater trend to improvement early on. Authors also observed significant increase in FDG uptake on PET scan. Positive results, however, were tempered by the inability to compare treatment groups due to small sample size and the inconsistency of improvement. Though some patients improved, nearly as many showed no change and some even declined in functional status. Attempts to establish efficacy after bone marrow transplantation ran into similar

problems. Though improvement was noted in some measures of functional deficit (namely, motor), results of other functional tests were not as positive. Additionally, neuropsychological testing suggested possible improvement but provided mixed results that were difficult to interpret.

Given the nature of early phase clinical trials, small sample size was a limitation in all of the studies described; particularly with regard to small, randomized trials, it is difficult to draw conclusions from comparisons of intervention and control groups. Additionally, uncertainties regarding optimal patient characteristics, intervention, and study design abound. Despite some initially promising results, the success of cell therapy for stroke is determined by a myriad of factors, including stroke characteristics (anatomy, type, timing), cell type and mechanism of action, and technical considerations, such as method of delivery, dose, choice of outcome measure, and use of immunosuppression (Bliss et al. 2007; Locatelli et al. 2009; Savitz et al. 2004), none of which have been optimized in clinical applications.

Thus far, all clinical trials of intracerebral cell therapy have been in "chronic" stroke patients or those who continue to have functional deficit for greater than 3 months following onset of stroke. Choice of such patients is likely one of practicality; given the invasive nature of the procedure, safety trials of direct intraparenchymal cell delivery require stable patients with no reasonable expectation of spontaneous natural recovery. Translation of data from preclinical models to chronic stroke patients, however, is problematic. Particularly without strong primate data, extrapolation of results from rat studies is difficult, given the predominant use of allografted human cells, concern for immune rejection, and short rodent lifespan (Kalladka and Muir 2011).

Clinical studies of cell therapy for stroke remain in the nascent phase. As preclinical work evolves, with identification of promising cell types and further elucidation of mechanism of action, so too will translation to the clinical realm. Ongoing trials, such as with SB623 cells, will continue to shed light on the ability of intracranial cell delivery to safely translate into the clinical realm and convey therapeutic benefit. Intracerebral delivery of cells remains a viable and promising option, with great potential for future clinical endeavors.

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