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Cell Therapy for Stroke: Review of Previous Clinical Trials and Introduction of Our New Trials

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Abstract

Stroke is still a leading cause of death and disability, and despite intensive research, few treatment options exist. A recent breakthrough in cell therapy is expected to reverse the neurological sequelae of stroke. Although some pioneer studies on the use of cell therapy for the treatment of stroke have been reported, certain problems still remain unsolved. We investigated the use of autologous bone marrow stromal cell (BMSC) transplantation for the treatment of stroke, to develop it as the next-generation cell therapy. In this study, we introduce the preparation of a new clinical trial, the Research on Advanced Intervention using Novel Bone marrow stem cell (RAINBOW) study. The trial will start in 2016, and we hope that it will not only be helpful for treating patients but also for clarifying the therapeutic mechanisms. Moreover, we review stem cell therapeutics as an emerging paradigm in stroke (STEPS) and the guidelines for the development of cell therapy for stroke in the United States as well as introduce the development of new guidelines in Japan. These guidelines are expected to encourage the development of cell therapy for stroke management.

Key words: bone marrow stromal cells, stroke, cell therapy, regenerative medicine

Introduction

Stroke is still a leading cause of death and disability,[1)] but few treatment options exist despite intensive research. Once the central nervous system (CNS) is damaged, it is difficult for the tissue to regenerate. Because regenerative medicine has rapidly progressed in the recent years, a breakthrough is expected that can reverse the neurological sequelae, which are currently difficult to cure. The therapeutic potential of cell transplantation has been demonstrated in various pathological conditions involving CNS, including traumatic brain injury,[2,3)] traumatic spinal cord injury,[4–8)] degenerative disease,[9)] demyelinating disease[10)] and ischemic stroke.[11–15)]

Various cell sources, each with different characteristics, can be used in cell therapy. Among somatic stem cells, the mesenchymal stem cell (MSC, also known as bone marrow stromal cell [BMSC]) began attracting much attention after Azizi et al. (1998) reported that MSCs could differentiate into neural cells.[16)] MSC has some advantages for clinical use, such as simple, established cell collection and culture methods; availability of autologous cell source; and fewer problems than the pluripotent stem cells, such as embryonic stem cells (ESC) and the induced pluripotent stem cells (iPSC) with respect to bioethics, immunoreaction, and tumorigenesis. Furthermore, MSCs have various origins, such as bone marrow, fat tissue, dental pulp, and umbilical cord.[17)]

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Review of Pioneer Clinical Trials of Cell Therapy

In 2005, some research groups reported pioneer studies on the use of cell therapy for stroke. Kondziolka et al. reported a phase 2 trial with LBS-neurons (human teratocarcinoma cell line origin, Layton BioScience, Inc.).[18)] They tested the usefulness of neuronal cell transplantation in patients with substantial fixed motor deficits associated with a basal ganglia stroke. Regrettably, there was no significant change in the motor score of patients who received cell implants compared with that of controls. Serial evaluations demonstrated that three patients experienced complications: one with a single seizure, one with syncope, and one with an asymptomatic chronic subdural hematoma.

In the same year, Savitz et al. reported an open-label trial of stereotactic transplantation with LGE

cells (fetal porcine striatum-derived cells, Genvec, Inc.) in five patients with basal ganglia infarcts and stable neurological deficits.[19] Two patients showed improvement in speech, language, and/or motor impairments, but two patients experienced adverse effects; one experienced temporary worsening of motor deficits 3 weeks after transplantation and the other experienced seizures 1 week after transplantation. The study was terminated by the U.S. Food and Drug Administration (FDA).

In Korea, on the other hand, Bang et al. reported feasibility and safety of cell therapy using intravenous infusion of autologous MSCs in 2005.[20] They treated five patients with cerebral infarcts within the middle cerebral arterial (MCA) territory and with severe neurological deficits. They reported that serial evaluations showed no adverse effects. Outcomes temporarily improved in MSC-treated patients compared with the controls, but there was no significant change in the motor scores at 12 months.

New Generation of Cell Therapy for Stroke: the Research on Advanced Intervention using Novel Bone marrow Stem Cell (RAINBOW) Study

Although the failure of these pioneer studies disappointed people who had high hopes for regenerative medicine, the researchers for new cell therapy were aware of the unsolved problems. We aimed to evolve autologous BMSC transplantation for stroke into the next generation.

We have reported the results of translational research on BMSC transplantation for stroke. Human BMSCs were cultured with allogeneic human platelet lysate (hPL) instead of fetal calf serum (FCS).[21–23] The cells were injected stereotactically into rat ischemic brains.[21,22,24] The donor cells were labeled in advance with superparamagnetic iron oxide (SPIO) for cell tracking using magnetic resonance imaging (MRI).[22,25,26] After the transplantation, ^{18}F-fluorodeoxyglucose positron emission tomography (FDG-PET) and ^{123}I-iomazenil single-photon emission computed tomography (IMZ-SPECT) were performed for the analysis of cellular function and metabolism.[27,28] There was no difference in the surface markers and cell proliferation between hPL and FCS.[23] Although a rotarod test showed that motor function deteriorated in rats suffering from permanent MCA occlusion, BMSC-hPL transplantation significantly enhanced recovery of motor function.[21] MRI demonstrated that SPIO–BMSCs aggressively migrated toward the lesion.[22,26] Moreover, FDG-PET and IMZ-SPECT showed that BMSC transplantation promoted the recovery of glucose utilization and the binding potential of iomazenil in the peri-infarct area, respectively.[27,28] Histological analysis supported the MRI findings and showed the inclination of donor cells for neural differentiation.[22,26] We concluded that allogeneic hPL may be valuable and safe for expanding BMSCs. The application of bioimaging techniques is also valuable for BMSC transplantation for stroke.

We translated these results to the optimal design of a new clinical trial called the RAINBOW study, which is a phase 1 study for acute ischemic stroke patients (Table 1). Autologous BMSCs are cultured with allogeneic hPL in the cell-processing center (Fig. 1) and labeled with SPIO. They are then stereotactically transplanted around the infarct (Fig. 2). After the transplantation, MRI for cell tracking, FDG-PET, and IMZ-SPECT are performed to analyze the therapeutic effects. The trials will start in 2016, and we hope that it will be helpful not only for the patients but also for clarifying the underlying therapeutic mechanisms.

Table 1 Summary of the protocol for the RAINBOW study

Autologous bone marrow stromal cell transplantation for acute ischemic stroke	
Purpose	The primary purpose of the clinical study is to determine the safety of autologous bone marrow stromal cell HUNS001-01 when administered to acute ischemic stroke patients
Phase	Phase 1
Study design	Open label, uncontrolled, dose response study
Condition	Acute ischemic stroke, ICA territory
Intervention	HUNS001-01 will be administered around the infarct area stereotactically. Each patient in one of two groups will be given a dose of 20 or 50 million cells
Primary outcome measures	Safety (time frame: 1 year)
Secondary outcome measures	Improvement in stroke symptoms and functional shift in bio-imaging (time frame: 1 year). Possible improvement in stroke symptoms will be determined by a variety of neurological assessments. Possible functional shift will be assessed using MRI, FDG-PET, and IMZ-SPECT
Estimated enrollment	≥6 (low-dose group: 3, high-dose group 3)

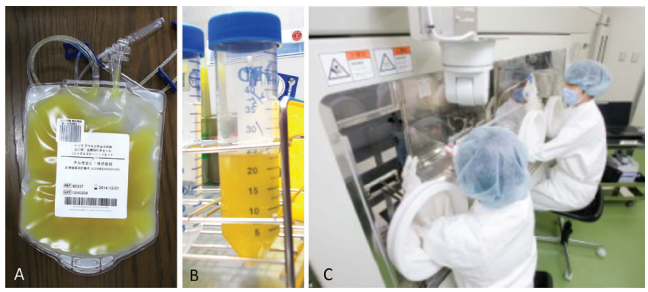

Fig. 1 Autologous BMSC culture with hPL in GMP level. Panels A and B show a bag containing human platelet derived from healthy volunteers (A) and hPL which was made at CPC (B). Panel C shows human BMSC culture with allogeneic PL in CPC corresponding to GMP.

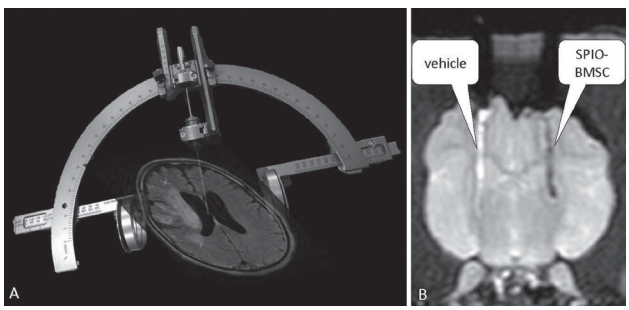

Fig. 2 Stereotactic transplant of autologous BMSC labeled with SPIO. Panel A shows that autologous BMSC are transplanted around the infarct stereotactically. Panel B shows T_2-weighted image of a decapitated pig brain by 3 Tesla-MRI for clinical use. The image indicates strong contrast of the needle track with injected SPIO–BMSC (*black*) to the pig brain parenchyma (*gray*) or the vehicle (*white*).

Guidelines Encourage Development of Cell Therapy for Stroke

After the failure of the pioneer studies in 2005, some researchers in the United States intended to establish guidelines for the development of cell therapy for stroke. In 2007, the stem cell therapeutics as an emerging paradigm in stroke (STEPS) group, the members of which belonged to academia, industry, and the National Institute of Health (NIH), launched a new standard to develop the cell therapy. The first recommendation, STEPS-I, was published in 2009[29] and the guidelines included the design of the pre-clinical study as well as that of the early phase of clinical trials. In 2011, STEPS-II was published with the participation of FDA as part of the working group.[30] In 2014, STEPS-III was published and included the most recent guidelines regarding the design of the later phase of clinical trials.[31]

Since the series of STEPS guidelines were published, many clinical trials using cell therapy for stroke have started not only in the United States but also in other countries throughout the world. STEPS members also started new trials in rapid succession. At the International Stroke Conference 2014 (San Diego, CA), Steinberg et al. reported a phase 1/2A study with SB623 cells (SanBio, Inc, CA). The cell source was genetically modified bone marrow cells. Eighteen patients with ischemic stroke underwent the stereotactic transplantation in the chronic phase. It was noteworthy that they showed the potential to improve motor function. Hess et al. also reported a study using MultiStem (Athersys Inc, Cleveland) at the European Stroke Organization Conference 2015 (Glasgow, UK). The cell source was allogeneic bone marrow-derived cells, and 126 patients with ischemic stroke underwent intravenous transplantation in the acute phase. They showed favorable recovery within an early therapeutic time window (24–36 h after stroke).

To date, there are only two completed trials using autologous bone marrow-derived cells in Japan: a phase 2 trial using autologous MSCs by Honmou et al.[32] and a phase 1/2A trial with autologous bone marrow-derived mononuclear cells by Taguchi et al.[33] Compared with previous basic research on stem cells, such as the establishment of iPSC,[34] these numbers seem too low. To encourage the translation of basic science into clinical situations, Japanese developers and regulatory agencies need to think on how to proceed. In 2012, the Ministry of Health, Labour and Welfare in Japan started a new project, Initiative for Accelerating Regulatory Science in Innovative Drugs, Medical Devices, and Regenerative Medicine. The project promoted creating guidelines for the development of new drugs, medical devices, and cell products. As a part of the project, the working group (the chairman is Dr. Kiyohiro Houkin, Hokkaido University, Sapporo, Japan) to develop new guidelines for cell therapy for stroke started in November 2013. The members included neurosurgeons, neurologists, a neuroradiologist, a physician for neurorehabilitation, basic scientists, regulatory scientists, and Pharmaceuticals and Medical Devices Agency (PMDA) staff. It was important to take an original stance in Japan because of domestic regulations for regenerative medicine, although the STEPS series was considered as a reference.[29–31] The scope of the guidelines was the use of cell therapy for ischemic stroke. The cell sources were somatic stem cells, for example, MSCs, MNCs, and neural stem cells. It was noteworthy that not only the developers but also PMDA could use the guidelines in reviews. In 2016, the guidelines in Japanese will be launched, and then the text will be translated

into English to disseminate all over the world. It is expected that the new guidelines will promote the development of new cell therapies in Japan and will be established as an aspect of stroke management in the future.

Conflict of Interest Disclosure

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References

1) Goldstein LB, Adams R, Alberts MJ, Appel LJ, Brass LM, Bushnell CD, Culebras A, Degraba TJ, Gorelick PB, Guyton JR, Hart RG, Howard G, Kelly-Hayes M, Nixon JV, Sacco RL; American Heart Association/American Stroke Association Stroke Council; Atherosclerotic Peripheral Vascular Disease Interdisciplinary Working Group; Cardiovascular Nursing Council; Clinical Cardiology Council; Nutrition, Physical Activity, and Metabolism Council; Qualityof Care and Outcomes Research Interdisciplinary Working Group; American Academy of Neurology: Primary prevention of ischemic stroke: a guideline from the American Heart Association/American Stroke Association Stroke Council: cosponsored by the Atherosclerotic Peripheral Vascular Disease Interdisciplinary Working Group; Cardiovascular Nursing Council; Clinical Cardiology Council; Nutrition, Physical Activity, and Metabolism Council; and the Quality of Care and Outcomes Research Interdisciplinary Working Group: the American Academy of Neurology affirms the value of this guideline. *Stroke* 37: 1583–633, 2006
2) Lu D, Mahmood A, Wang L, Li Y, Lu M, Chopp M: Adult bone marrow stromal cells administered intravenously to rats after traumatic brain injury migrate into brain and improve neurological outcome. *Neuroreport* 12: 559–563, 2001
3) Mahmood A, Lu D, Wang L, Li Y, Lu M, Chopp M: Treatment of traumatic brain injury in female rats with intravenous administration of bone marrow stromal cells. *Neurosurgery* 49: 1196–1203; discussion 1203–1194, 2001
4) Chopp M, Zhang XH, Li Y, Wang L, Chen J, Lu D, Lu M, Rosenblum M: Spinal cord injury in rat: treatment with bone marrow stromal cell transplantation. *Neuroreport* 11: 3001–3005, 2000
5) Ankeny DP, McTigue DM, Jakeman LB: Bone marrow transplants provide tissue protection and directional guidance for axons after contusive spinal cord injury in rats. *Exp Neurol* 190: 17–31, 2004
6) Ohta M, Suzuki Y, Noda T, Ejiri Y, Dezawa M, Kataoka K, Chou H, Ishikawa N, Matsumoto N, Iwashita Y, Mizuta E, Kuno S, Ide C: Bone marrow stromal cells infused into the cerebrospinal fluid promote functional recovery of the injured rat spinal cord with reduced cavity formation. *Exp Neurol* 187: 266–278, 2004
7) Yano S, Kuroda S, Lee JB, Shichinohe H, Seki T, Ikeda J, Nishimura G, Hida K, Tamura M, Iwasaki Y: *In vivo* fluorescence tracking of bone marrow stromal cells transplanted into a pneumatic injury model of rat spinal cord. *J Neurotrauma* 22: 907–918, 2005
8) Sykova E, Jendelova P, Urdzikova L, Lesny P, Hejcl A: Bone marrow stem cells and polymer hydrogels: two strategies for spinal cord injury repair. *Cell Mol Neurobiol* 26: 1113–1129, 2006
9) Li Y, Chen J, Wang L, Zhang L, Lu M, Chopp M: Intracerebral transplantation of bone marrow stromal cells in a 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine mouse model of Parkinson's disease. *Neurosci Lett* 316: 67–70, 2001
10) Sasaki M, Honmou O, Akiyama Y, Uede T, Hashi K, Kocsis JD: Transplantation of an acutely isolated bone marrow fraction repairs demyelinated adult rat spinal cord axons. *Glia* 35: 26–34, 2001
11) Chen J, Li Y, Wang L, Lu M, Zhang X, Chopp M: Therapeutic benefit of intracerebral transplantation of bone marrow stromal cells after cerebral ischemia in rats. *J Neurol Sci* 189: 49–57, 2001
12) Shichinohe H, Kuroda S, Lee JB, Nishimura G, Yano S, Seki T, Ikeda J, Tamura M, Iwasaki Y: *In vivo* tracking of bone marrow stromal cells transplanted into mice cerebral infarct by fluorescence optical imaging. *Brain Res Brain Res Protoc* 13: 166–175, 2004
13) Borlongan CV, Lind JG, Dillon-Carter O, Yu G, Hadman M, Cheng C, Carroll J, Hess DC: Bone marrow grafts restore cerebral blood flow and blood brain barrier in stroke rats. *Brain Res* 1010: 108–116, 2004
14) Iihoshi S, Honmou O, Houkin K, Hashi K, Kocsis JD: A therapeutic window for intravenous administration of autologous bone marrow after cerebral ischemia in adult rats. *Brain Res* 1007: 1–9, 2004
15) Miki Y, Nonoguchi N, Ikeda N, Coffin RS, Kuroiwa T, Miyatake S: Vascular endothelial growth factor gene-transferred bone marrow stromal cells engineered with a herpes simplex virus type 1 vector can improve neurological deficits and reduce infarction volume in rat brain ischemia. *Neurosurgery* 61: 586–594; discussion 594–585, 2007
16) Azizi SA, Stokes D, Augelli BJ, DiGirolamo C, Prockop DJ: Engraftment and migration of human bone marrow stromal cells implanted in the brains of albino rats: similarities to astrocyte grafts. *Proc Natl Acad Sci U S A* 95: 3908–3913, 1998
17) Dominici M, Le Blanc K, Mueller I, Slaper-Cortenbach I, Marini F, Krause D, Deans R, Keating A, Prockop Dj, Horwitz E: Minimal criteria for defining multipotent mesenchymal stromal cells: the International

Society for Cellular Therapy position statement. *Cytotherapy* 8: 315–317, 2006
18) Kondziolka D, Steinberg GK, Wechsler L, Meltzer CC, Elder E, Gebel J, Decesare S, Jovin T, Zafonte R, Lebowitz J, Flickinger JC, Tong D, Marks MP, Jamieson C, Luu D, Bell-Stephens T, Teraoka J: Neurotransplantation for patients with subcortical motor stroke: a phase 2 randomized trial. *J Neurosurg* 103: 38–45, 2005
19) Savitz SI, Dinsmore J, Wu J, Henderson GV, Stieg P, Caplan LR: Neurotransplantation of fetal porcine cells in patients with basal ganglia infarcts: a preliminary safety and feasibility study. *Cerebrovasc Dis* 20: 101–107, 2005
20) Bang OY, Lee JS, Lee PH, Lee G: Autologous mesenchymal stem cell transplantation in stroke patients. *Ann Neurol* 57: 874–882, 2005
21) Sugiyama T, Kuroda S, Takeda Y, Nishio M, Ito M, Shichinohe H, Koike T: Therapeutic impact of human bone marrow stromal cells (hBMSC) expanded by animal serum-free medium for cerebral infarct in rats. *Neurosurgery* 68: 1733–1742, 2011
22) Ito M, Kuroda S, Sugiyama T, Shichinohe H, Takeda Y, Nishio M, Koike T, Houkin K: Validity of bone marrow stromal cell expansion by animal serum-free medium for cell transplantation therapy of cerebral infarct in rats: a serial MRI study. *Transl Stroke Res* 2: 294–306, 2011
23) Shichinohe H, Kuroda S, Sugiyama T, Ito M, Kawabori M, Nishio M, Takeda Y, Koike T, Houkin K: Biological features of human bone marrow stromal cells (hBMSC) cultured with animal protein-free medium-safety and efficacy of clinical use for neurotransplantation. *Transl Stroke Res* 2: 307–315, 2011
24) Kawabori M, Kuroda S, Sugiyama T, Ito M, Shichinohe H, Houkin K, Kuge Y, Tamaki N: Intracerebral, but not intravenous, transplantation of bone marrow stromal cells enhances functional recovery in rat cerebral infarct: an optical imaging study. *Neuropathology* 32: 217–226, 2012
25) Shichinohe H, Kuroda S, Kudo K, Ito M, Kawabori M, Miyamoto M, Nakanishi M, Terae S, Houkin K: Visualization of the superparamagnetic iron oxide (SPIO)-labeled bone marrow stromal cells using a 3.0-T MRI: a pilot study for clinical testing of neurotransplantation. *Transl Stroke Res* 3: 99–106, 2012
26) Shichinohe H, Yamauchi T, Saito H, Houkin K, Kuroda S: Bone marrow stromal cell transplantation enhances recovery of motor function after lacunar stroke in rats. *Acta Neurobiol Exp* (*Wars*) 73: 354–363, 2013
27) Miyamoto M, Kuroda S, Zhao S, Magota K, Shichinohe H, Houkin K, Kuge Y, Tamaki N: Bone marrow stromal cell transplantation enhances recovery of local glucose metabolism after cerebral infarction in rats: a serial 18F-FDG PET study. *J Nucl Med* 54: 145–150, 2013
28) Saito H, Magota K, Zhao S, Kubo N, Kuge Y, Shichinohe H, Houkin K, Tamaki N, Kuroda S: 123I-iomazenil single photon emission computed tomography visualizes recovery of neuronal integrity by bone marrow stromal cell therapy in rat infarct brain. *Stroke* 44: 2869–2874, 2013
29) STEPS participants: Stem cell therapies as an emerging paradigm in stroke (STEPS): bridging basic and clinical science for cellular and neurogenic factor therapy in treating stroke. *Stroke* 40: 510–515, 2009
30) Savitz SI, Chopp M, Deans R, Carmichael ST, Phinney D, Wechsler L: Stem cell therapy as an emerging paradigm for stroke (STEPS) II. *Stroke* 42: 825–829, 2011
31) Savitz SI, Cramer SC, Wechsler L, Consortium S: Stem cells as an emerging paradigm in stroke 3: enhancing the development of clinical trials. *Stroke* 45: 634–639, 2014
32) Honmou O, Houkin K, Matsunaga T, Niitsu Y, Ishiai S, Onodera R, Waxman SG, Kocsis JD: Intravenous administration of auto serum-expanded autologous mesenchymal stem cells in stroke. *Brain* 134(Pt 6): 1790–1807, 2011
33) Taguchi A, Sakai C, Soma T, Kasahara Y, Stern DM, Kajimoto K, Ihara M, Daimon T, Yamahara K, Doi K, Kohara N, Nishimura H, Matsuyama T, Naritomi H, Sakai N, Nagatsuka K: Intravenous autologous bone marrow mononuclear cell transplantation for stroke: phase 1/2a clinical trial in a homogeneous group of stroke patients. *Stem Cells Dev* 24: 2207–2218, 2015
34) Takahashi K, Yamanaka S: Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell* 126: 663–676, 2006

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