

Current Opinion of Bone Marrow Stromal Cell Transplantation for Ischemic Stroke

Satoshi KURODA¹

¹Department of Neurosurgery, Graduate School of Medicine and Pharmaceutical Science, University of Toyama, Toyama, Toyama

Abstract

This article reviews recent advancement and perspective of bone marrow stromal cell (BMSC) transplantation for ischemic stroke, based on current information of basic and translational research. The author would like to emphasize that scientific approach would enable us to apply BMSC transplantation into clinical situation in near future.

Key words: bone marrow, stem cell, transplantation, ischemic stroke, clinical trial

Introduction

For these three decades, cell therapy has been expected to promote functional recovery after central nervous system (CNS) disorders, including ischemic stroke. A variety of cells have been studied as the candidate donor cells for this purpose. These include embryonic stem (ES) cells, neural stem cells, inducible pluripotent stem (iPS) cells, and bone marrow stromal cells (BMSCs). Of these, the BMSCs may have the most promising potential among them, because they can be obtained from the patients themselves and easily expanded without posing any ethical and immunological problems. Based on recent observations, the transplanted BMSCs significantly enhance functional recovery after the insults in animal models of ischemic stroke. Some preliminary clinical tests have been conducted for patients with ischemic stroke in the world.^{1,2)} This review article briefly summarizes recent advances and perspective of BMSC transplantation for ischemic stroke.

Biological Features of BMSC

The BMSCs are non-hematopoietic cells and one of the major mesenchymal stem cells in humans. Originally, they were isolated from the bone marrow as adherent, fibroblast-like-shaped cells. The BMSCs are known to have the ability for multi-lineage differentiations, immuno-modulation, and hematopoietic support (Fig. 1). Based on these unique biological

features, the BMSCs have been widely expected as a transplantable resource for cell therapy.³⁾ The BMSCs regulate the proliferation and differentiation of hematopoietic cells in the bone marrow under physiological condition. However, there is increasing evidence that the BMSCs are playing a key role in the homeostasis/turnover of peripheral tissues and, if needed, could be mobilized from the bone marrow into the circulating blood during tissue injury and stress, facilitating the regeneration of damaged organs.³⁾ Therefore, the BMSCs are believed to maintain the homeostasis in the whole body as well as in the bone marrow.

Recent studies have rapidly clarified the mechanisms through which the transplanted BMSCs enhance functional recovery after ischemic stroke. It is well known that the BMSCs aggressively migrate into the injured tissues *in vitro* and *in vivo*. Chemokine system may be involved in their migration capacity into cerebral infarct. Of these, CXCR4 on the surface of BMSCs, a specific receptor for stromal cell-derived factor (SDF)-1a, is reported to play an important role in their migration in the CNS. The BMSCs harvested from the wild-type mice, but not CXCR4-deficient mice, migrate towards peri-infarct area when directly transplanted into the mice infarct brain.⁴⁾ There are few studies whether the engrafted BMSCs retain their proliferative activity in the host brain or not. Yano et al. (2005) labeled the green fluorescence protein (GFP) expressing BMSCs with a superparamagnetic iron oxide (SPIO) agent and transplanted into the ipsilateral striatum of the mice infarct brain (“double labeling” technique) and

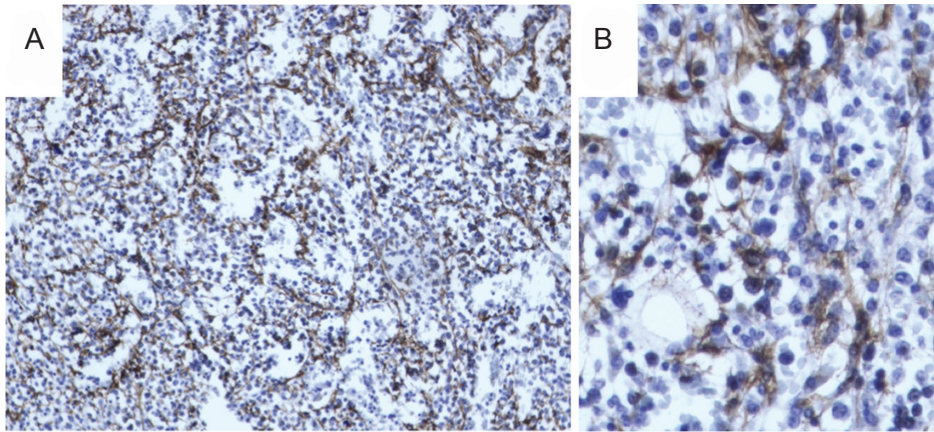


Fig. 1 Distribution of CD90-positive cells in the human bone marrow. CD90 is an immunohistochemical marker for the non-hematopoietic cells. The CD90-positive cells morphologically simulate the fibroblasts. Original magnification; $\times 40$ (A) and $\times 200$ (B). BMSC: bone marrow stromal cell.

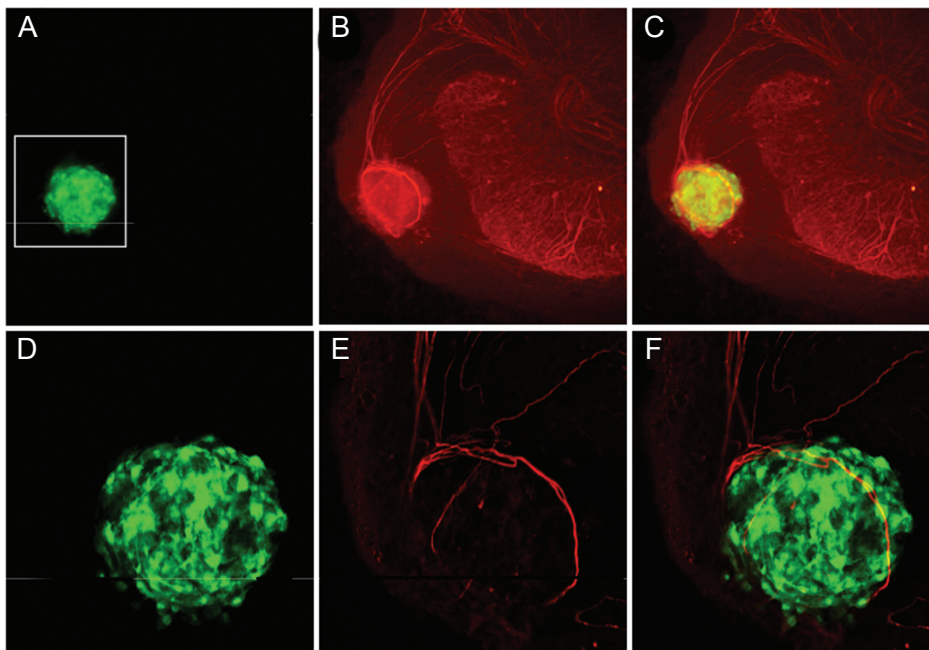


Fig. 2 Photomicrograph of fluorescence immunohistochemistry 3 weeks after GFP-BMSC transplantation, showing that the transplanted BMSC survived on the spinal cord slice (green, A) and promoted the SMI32-positive neurite outgrowth from the host neurons (red, B). Their merged image is shown in panel C. *White box* (A) indicates field-of-view for panels D–F. Confocal laser microscopy shows that the extended neurites (red, E) get tangled around the BMSC that migrated into the slice like a “yoyo” (green, D). Their merged image is shown in panel F.¹³ BMSC: bone marrow stromal cell.

found that the BMSCs actively proliferate, migrate toward the lesion, and partially express the neuronal phenotype in the host brain after transplantation.⁵

Nowadays, the BMSCs are known to produce some neuroprotective or neurotrophic factors and support the survival of the host neural cells.⁶ This hypothesis is readily reasonable because the BMSC per se support the homing, proliferation, and differentiation of the hematopoietic cells in the bone marrow by producing a variety of cytokines.⁷ Interestingly, the conditioned medium of BMSCs significantly promote neurite outgrowth from the dorsal root ganglion.⁸ They also release soluble neuroprotective factors, including nerve growth factor (NGF), hepatocyte growth factor (HGF), and brain-derived neurotrophic factor (BDNF), and significantly ameliorate glutamate-induced damage of neurons.⁹ The BMSCs markedly

promote the neurite extension from the neurons in the organotypic slice of the brain and spinal cord (Fig. 2).^{10,11} The BMSCs also protect the neurovascular integrity between basement membrane and astrocyte end-feet and ameliorate brain damage in stroke-prone spontaneous hypertensive rats.¹² Finally, Shichinohe et al. demonstrated that the BMSCs serve a “nursing effect” to the damaged neurons and activate the neural stem cells in the host brain by producing neurotrophic factors. Of these, BDNF is considered to be the most powerful factor to protect and repair the damaged neurons.¹³ Therefore, the transplanted BMSCs trigger endogenous signaling pathways of survival and repair in neurons by secreting soluble neurotrophic factors.

Both neutrophils and macrophages are well known to play an important role in the early inflammation after cerebral infarct.¹⁴ Indeed, their inflammatory

response may be an essential process to clear cellular debris and initiate the healing pathways. Simultaneously, however, these inflammatory reactions may also give rise to cytotoxic damage to the surviving neurons, astrocytes, and endothelial cells in the peri-infarct area.¹⁴⁾ The BMSCs have currently been investigated as donor cells for novel cell therapy to prevent and to treat clinical disease associated with aberrant immune response. In the host, the BMSCs may attenuate pro-inflammatory cytokine and chemokine induction and reduce pro-inflammatory cell migration into sites of injury and infection.¹⁵⁾ Therefore, the transplanted BMSCs may prevent excessive inflammatory response and prevent further tissue damage in the peri-infarct area through their immuno-modulatory capacity.

The BMSCs are believed to differentiate into neural cells in the host's brain. This theory is based on the findings that BMSC simulate neuronal morphology and express the proteins specific for neurons *in vitro*^{16,17)} or *in vivo*.^{18,19)} It may sound strange that the BMSC have the ability to differentiate into the neural cells. However, the BMSC *per se* express the genes related to neuronal and glial cells.²⁰⁾ Recent studies also show that the BMSCs can alter their gene expression profile in response to exogenous stimuli and increase the genes related to the neural cells.^{20–22)} Sanchez-Ramos et al. (2000) showed that a small fraction of BMSCs cultured in epidermal growth factor (EGF) or retinoic acid/BDNF expressed nestin, NeuN, or GFAP, and that the proportion of NeuN-expressing cells increased when BMSCs were co-cultured with fetal mouse midbrain neurons.¹⁶⁾ Wislet-Gendebien et al. (2005) co-cultured the BMSCs with cerebellar granule cells and assessed their fates. They found that the nestin-expressing BMSCs express other neuronal markers and that BMSC-derived neuron-like cells fire single-action potentials in response to neurotransmitters such as glutamate.²³⁾ Hokari et al. (2008) also demonstrated that a certain subpopulation of the BMSCs morphologically simulated the neuron and expressed the neuron-specific proteins without any evidence of cell fusion, when co-cultured with the neurons.⁹⁾ These findings strongly suggest that at least a certain subpopulation of the BMSCs have the potential to alter their gene expression profile and to differentiate into the neural cells in response to the surrounding environment. More importantly, the findings indicate that only the subgroup of BMSCs with potential of neural differentiation can survive in the host brain for a long time (> 4 weeks). According to recent work by Liu et al., the BMSC may enhance the axonal sprouting from the survived cortical neurons in the peri-infarct area.²⁴⁾ Chiba et al. have also found that the BMSCs are integrated

into the neural circuits of the host spinal cord and promote functional recovery.²⁵⁾ These biological properties of BMSC may play a key role to enhance functional recovery after ischemic stroke.

Based on these observations, the exogenous transplantation of BMSCs is now believed to enhance functional recovery through multiple mechanisms, including nursing effect, anti-inflammatory action, and neural cell differentiation in patients with ischemic stroke. This speculation seems quite natural, because the BMSCs are isolated only from their adhesive characteristics and contain heterogeneous cell populations. Therefore, several cell subgroups of BMSCs contribute to functional recovery through each biological features. According to recent observations, the directly injected BMSCs rapidly migrate to the peri-infarct area within 2 weeks, serve the “nursing effect” to the ischemic brain for approximately 1 month, and start to exert regenerative capacity by differentiating into the neural cells thereafter (Fig. 3).^{13,26,27)}

How Should We Conduct Clinical Trial of BMSC Transplantation?

As described above, the observations in basic experiments are quite encouraging, and some clinical trials of BMSC transplantation have already been started for patients with ischemic stroke.^{28–30)} These results indicate that BMSC transplantation may be at least safe and feasible for patients with ischemic stroke. However, there are many variables that may affect the efficacy of BMSC transplantation in clinical setting. Thus, they include donor cell factors (safety, autologous or allogeneic, *ex vivo* cell expansion), patient factors (age, stroke type), treatment factors (interval since onset, delivery route, cell dose), and validation factors (neurological assessment, imaging).^{31,32)}

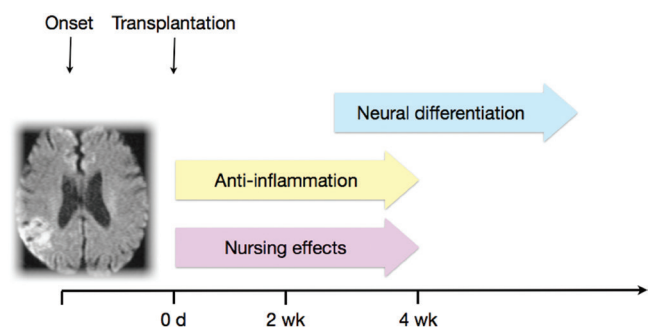


Fig. 3 The diagram shows the concept of multi-functional involvement in infarct brain by the BMSC. The BMSCs are heterogeneous cell populations and contribute to functional recovery through multiple mechanisms at different timing. BMSC: bone marrow stromal cell.

Allogeneic cells can permit “off the shelf” use even within 24 hours after the onset, but critical managements would be essential to maintain its safety. Autologous BMSC from patients themselves would be ideal as the donor cells for restorative medicine, but require several weeks for *ex vivo* expansion. Therefore, it should be scientifically proven that the BMSC could enhance functional recovery after ischemic stroke even when transplanted several weeks after the onset. More importantly, it would be critical to establish the feasible protocol to “safely and rapidly” expand the BMSC. Thus, the BMSC have been cultured in the medium including fetal calf serum (FCS) in the majority of animal experiments and even clinical trials.²⁸⁾ However, the FCS carries the potential risk of prion, viral, or zoonoses contamination. Alternatively, autologous serum is employed to expand the BMSC, but may require a large amount of serum.²⁹⁾ Very recently, human platelet lysate (PL) is proven useful to expand the BMSC as an alternative substitute. The human BMSC expanded with the FCS-free, PL-containing medium retain their capacity of migration, survival, and differentiation, and significantly promote functional recovery when stereotactically transplanted into the infarct brain. Therefore, PL may be a clinically valuable and safe substitute for FCS in expanding the human BMSC to regenerate the infarct brain.^{26,33,34)}

The BMSC are transplanted within 24 hours or 7 days after the insults in the majority of animal studies, whereas they are usually transplanted several weeks (or even several months) after stroke onset in previous clinical trials.^{28–30)} In order to resolve this discrepancy between animal experiments and clinical tests, pharmacological modulation may be useful to promote *in vitro* proliferation of the cultured BMSC to shorten the interval between stroke onset and cell therapy. For example, granulocyte-colony stimulating factor (G-CSF) significantly enhances the proliferation and growth factor production of the cultured BMSC and accelerate functional recovery by BMSC transplantation into the infarct brain.³⁵⁾ Such pharmacological modulation would be essential in considering autologous stem cell therapy for older patients with ischemic stroke, because adult stem cells, including BMSC, suffer the effect of aging and reduce their self-renewal and differentiation capacity.³⁶⁾ Very recent study has also demonstrated that G-CSF significantly promotes the proliferative capacity of BMSC harvested from the aged rats.³⁷⁾ These observations should be taken into considerations when establishing the treatment protocol in clinical situation.

The BMSC can be transplanted through several routes, including direct, intravenous, intra-arterial,

and intrathecal delivery. Although direct, intracerebral, or stereotactic injection permits most efficient delivery of the donor cells to the damaged tissue, safety would be essential for patients with ischemic stroke. Intravenous or intrathecal transplantation is attractive because of its non-invasive, safe technique for the host CNS, but has been reported to result in less pronounced cell migration and functional recovery than direct cell transplantation.³⁸⁾ Indeed, Kawabori et al. transplanted the BMSC into the infarct brain directly or intravenously in 7 days after the insult, that is clinically relevant timing. As the results, they concluded that intravenous administration of BMSC has limited effectiveness at clinically relevant timing and intracerebral administration should be chosen for patients with ischemic stroke.³⁹⁾ Alternatively, the intra-arterial injection of BMSC may be valuable to non-invasively deliver them to the damaged CNS.^{40,41)} Therefore, optimal transplantation technique should be developed to serve maximally safe and efficient results. It is also important to determine effective cell dose for patients with ischemic stroke, although there are few studies that denote this issue. Recently, Kawabori et al. directly transplanted the BMSC (1×10^5 or 1×10^6) into the infarct brain in 1 week or 4 weeks after the insult. As the results, they found that earlier transplantation requires a smaller number of donor cells for beneficial effects.⁴²⁾ These observations strongly suggest the importance of timing, delivery route, and cell dose to yield therapeutic effects of BMSC transplantation for ischemic stroke. Similar translational research should thoroughly be conducted to establish the scientifically proven protocol prior to the start of clinical testing.

In addition, it would be essential to develop the techniques to serially and non-invasively track the fate of the transplanted cells in the host CNS. Cell tracking technique would also be important as a “biologically relevant end point.”⁴³⁾ Magnetic resonance (MR) imaging, nuclear imaging, and optical imaging are the candidate modalities. The donor cells can be identified on MR imaging by labeling with a superparamagnetic iron oxide (SPIO) agent.^{26,44)} On the other hand, optical imaging technique may also serve future technology to visualize the BMSC engrafted in the damaged CNS. Quantum dot (QD) emits near-infrared (NIR) fluorescence with longer wavelength (800 nm) that can easily penetrate the living tissue. Very recent study has shown that the QD-labeled BMSC can be clearly visualized under *in vivo* fluorescence imaging through the skull and scalp for at least 8 weeks when transplanted into the infarct brain of rats (Fig. 4).^{41,45)} In addition, imaging technology would be valuable to assess

the effects of BMSC transplantation on the function of host brain. ^{18}F -fluorodeoxyglucose (FDG) PET may be a useful tool to visualize the beneficial effects of BMSC transplantation for ischemic brain in clinical situation.⁴⁶⁾ Miyamoto et al. reported that direct BMSC transplantation improved glucose metabolism in the infarct brain, using micro-PET/computed tomography (CT) apparatus. ^{123}I -iomazenil is a radioactive ligand selective for the central type of benzodiazepine receptor and is known useful to visualize the neuronal integrity on single photon emission tomography (SEPCT), which is a more widely available apparatus in clinical situation than PET. Using ^{23}I -iomazenil SPECT, Saito et al. also reported that the BMSCs enhanced functional recovery by improving the neuronal integrity in the peri-infarct area, when directly transplanted into the infarct brain at clinically relevant timing.⁴⁷⁾

Based on these observations, therefore, we should conduct clinical trials of BMSC transplantation for ischemic stroke by scientifically evaluating each step of transplantation procedures, which would avoid

repeating previous footsteps of long-time history to develop neuroprotective agents (Fig. 5).⁴³⁾

Muse Cell: A New Paradigm for Cell Therapy

Very recently, Dezawa and co-workers successfully isolated stress-tolerant adult human stem cells from cultured skin fibroblasts and BMSCs. These cells can self-renew, express a set of genes associated with pluripotency, and differentiate into endodermal, ectodermal, and mesodermal cells both *in vitro* and *in vivo*. When transplanted into immunodeficient mice by local or intravenous injection, they were integrated into damaged skin, muscle, or liver and differentiated into cytokeratin 14-, dystrophin-, or albumin-positive cells in the respective tissues. Furthermore, they can be efficiently isolated as SSEA-3-positive cells. Unlike authentic ES cells, their proliferation activity is not very high and they do not form teratoma in immunodeficient mouse testes. The findings are quite attractive,

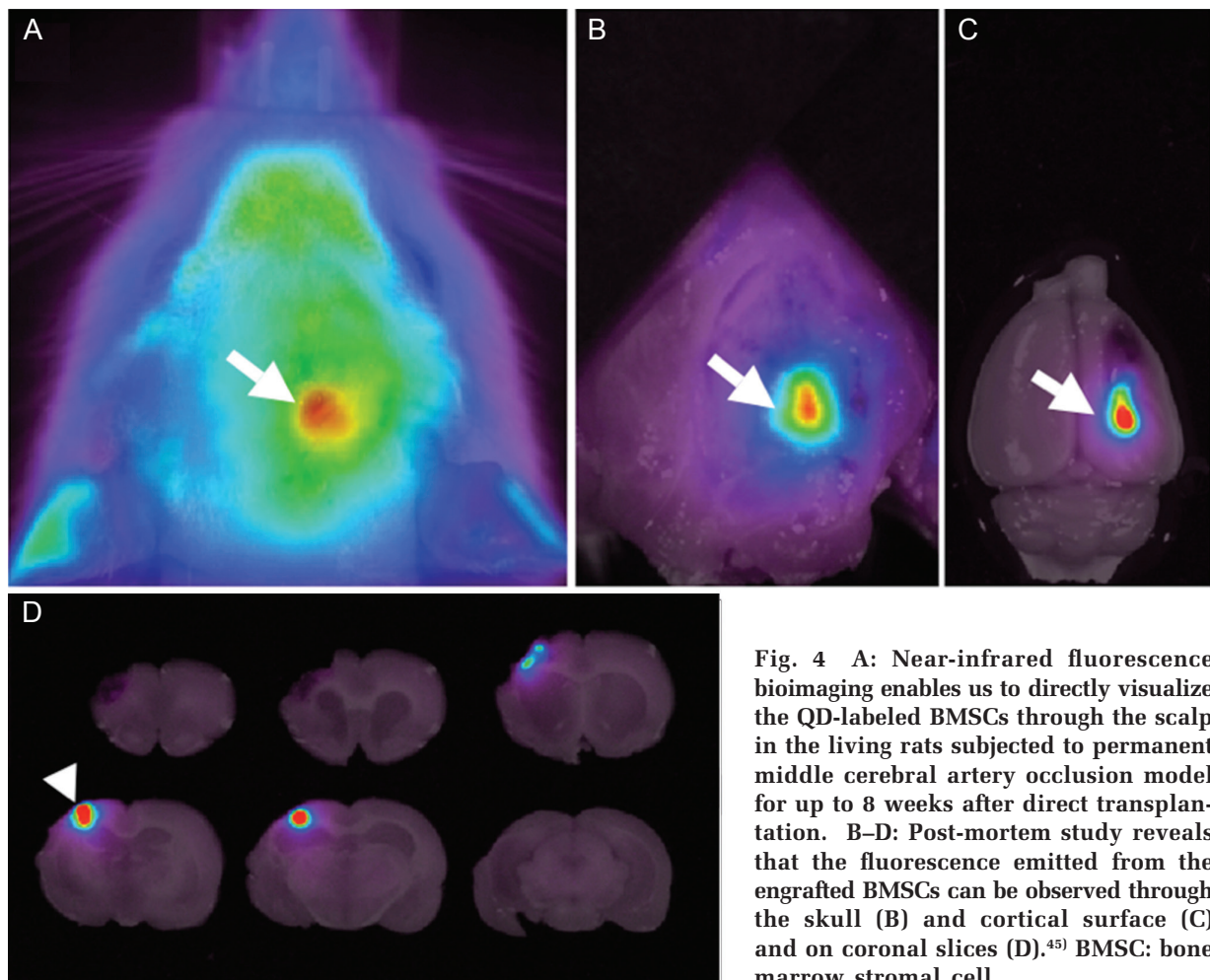


Fig. 4 A: Near-infrared fluorescence bioimaging enables us to directly visualize the QD-labeled BMSCs through the scalp in the living rats subjected to permanent middle cerebral artery occlusion model for up to 8 weeks after direct transplantation. B–D: Post-mortem study reveals that the fluorescence emitted from the engrafted BMSCs can be observed through the skull (B) and cortical surface (C) and on coronal slices (D).⁴⁵⁾ BMSC: bone marrow stromal cell.

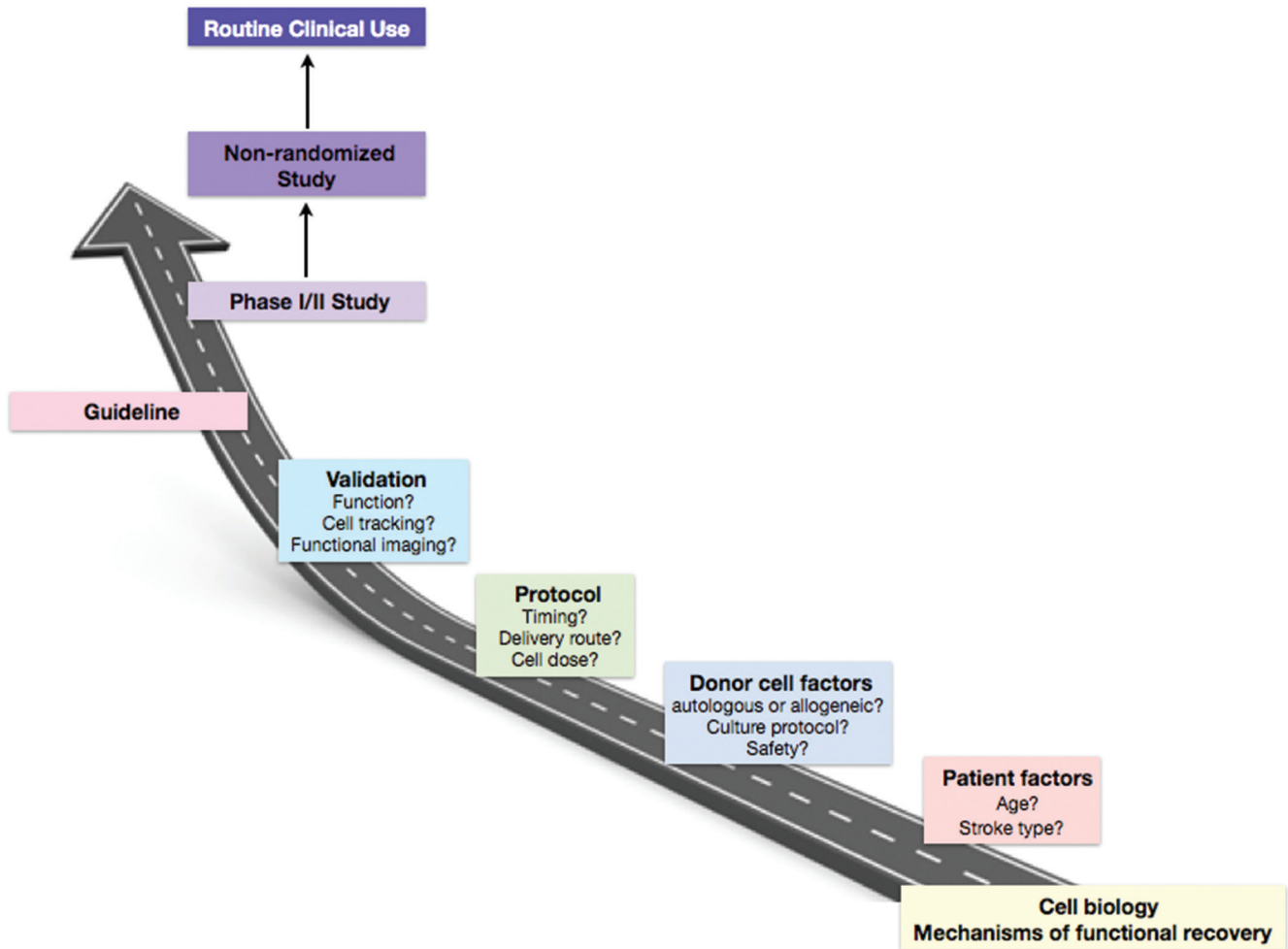


Fig. 5 The milestone for clinical application of BMSC transplantation for ischemic stroke. All the questions should be solved through “well-designed” preclinical studies and early-stage clinical trials.

because non-tumorigenic stem cells with the ability to generate multiple cell types of the three germ layers can be obtained through easily accessible adult human mesenchymal cells without introducing exogenous genes.⁴⁸⁾ These cells were named as multilineage-differentiating stress enduring (Muse) cells. Furthermore, they have proven that Muse cells are a primary source of iPS cells in human fibroblasts.⁴⁹⁾ The results strongly suggest that a certain subpopulation of BMSCs may have the biological properties of neural differentiation and contribute to regenerate the infarct brain.

Recent studies strongly suggest the possibility of Muse cells as biologically powerful stem cells for patients with ischemic stroke. Thus, they can survive in the infarct brain, differentiate into the neurons, and promote the recovery of motor function when directly engrafted into the murine model of ischemic stroke at clinically relevant timing (7 days) after the insult. A majority of engrafted Muse cells express

neuronal markers in the infarct brain at the peri-infarct area.²⁷⁾ Interestingly, motor function starts to improve at 5 weeks after Muse cell transplantation, which indicate that Muse cells require about 1 month for their migration, differentiation, and integration into the host brain. Furthermore, Muse cells are promptly committed to neural/neuronal-lineage cells when co-cultured with stroke brain slices *in vitro* and significantly improve motor function when directly injected into the rat brain subjected to middle cerebral artery occlusion at 2 days after the insult.⁵⁰⁾ Therefore, Muse cells, the unique and promising adult stem cell, are expected available for application into clinical situation for ischemic stroke through further studies.

Conclusion

This article reviews recent advancement of basic and translational research on BMSC transplantation for

ischemic stroke. The author believes that scientific approach would enable us to apply BMSC transplantation into clinical situation in near future.

Acknowledgments

This study was supported by a grant-in-aid from the Ministry of Education, Science, and Culture of Japan (No. 25293305).

Conflicts of Interest Disclosure

The author has reported no conflicts of interest.

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Address reprint requests to: Satoshi Kuroda, MD, PhD, Department of Neurosurgery, Graduate School of Medicine and Pharmaceutical Science, University of Toyama, 2630 Sugitani, Toyama, Toyama 930-0194, Japan.
e-mail: skuroda@med.u-toyama.ac.jp