Regenerative medicine for radiation emergencies

Yukihito Higashi1,2,*, Farina Mohamad Yusoff1, Shinji Kishimoto1 and Tatsuya Maruhashi1

1Department of Cardiovascular Regeneration and Medicine, Research Institute for Radiation Biology and Medicine, Hiroshima University, Japan
2Division of Regeneration and Medicine, Medical Center for Translational and Clinical Research, Hiroshima University Hospital, Japan
*Corresponding author. Department of Cardiovascular Regeneration and Medicine, Research Institute for Radiation Biology and Medicine, Hiroshima University, 1-2-3 Kasumi, Minami-ku, Hiroshima 734-8551, Japan. Tel: +81-82-257-5831; Fax: +81-82-257-5831; Email: yhigashi@hiroshima-u.ac.jp

(Received 29 July 2020; revised 25 August 2020; editorial decision 31 August 2020)

ABSTRACT

Hiroshima University is a ‘medical institution for tertiary radiation emergencies’ and a ‘medical support organization as a part of the International Atomic Emergency Agency Emergency Preparedness Response–Response and Assistance Network (IAEA EPR-RANET)’. To establish a system of regenerative medicine for radiation emergencies with treatment by implantation of various types of cells derived from induced pluripotent stem (iPS) cells, it is necessary to establish methods of defense against and treatment for radiation-induced damage from nuclear power plant accidents and nuclear terrorism. It is also necessary to develop cell therapy, cellular repair technology and regenerative biotechnology as regenerative medicine for radiation emergencies. Such applications have not been established yet. To develop a regenerative medical system, by using the existing one, for radiation emergencies, we will attempt to manage the cell-processing center to establish a safe and secured iPS cell bank for radiation medicine. By using this iPS cell bank as the central leverage, we will develop an education program for radiation emergency medicine and construct a network of regenerative medicine for radiation emergency medicine.

Keywords: regenerative medicine; radiation emergency; cell therapy; induced pluripotent stem cell; education program; network

INTRODUCTION

In Japan, radiation exposure accidents occurred in the Tokai-Mura criticality accident in September 1999 and in the Tokyo Electric Power Company Fukushima Dai-ichi Nuclear Power Plant accident in March 2011. Regenerative medicine treatment was not required for any workers in the Fukushima Dai-ichi Nuclear Power Plant accident. However, unfortunately, two workers who had been exposed to heavy doses of neutrons and gamma-rays in the Tokai-Mura criticality accident died of radiation-induced multiple organ failure despite treatment for multiple organ failure and repeated bone marrow transplantations [1, 2].

Hiroshima University is a ‘medical institution for tertiary radiation emergencies’ and a ‘medical support organization as a part of the International Atomic Emergency Agency Emergency Preparedness Response–Response and Assistance Network (IAEA EPR-RANET)’. Strategies for basic research and clinical research for radiation emergency medicine through international collaboration are being promoted. Various stem cells, including mesenchymal stem cells (MSCs), hematopoietic stem cells, endothelial progenitor cells (EPCs) and adipose tissue-derived stromal cells (ADRCs), are used for cell therapy in a clinical setting [3–6]. Beneficial effects of stem cell implantation on regeneration of tissues or organs have been shown. To establish a system of regenerative medicine for radiation emergencies, with treatment by implantation of various types of cells derived from induced pluripotent stem (iPS) cells, it is necessary to establish methods of defense against and treatment for radiation-induced damage from nuclear power plant accidents and nuclear terrorism and to develop cell therapy using various cells other than hematopoietic cells, cellular repair technology and regenerative biotechnology as regenerative medicine for radiation emergencies. Such applications have not been established yet. In order to develop a regenerative medical system, by using the existing one, for radiation emergencies, we will attempt to manage the cell processing center to establish a system for a safe and secured iPS cell bank for radiation medicine. By using this iPS cell bank as the central leverage, we will develop an education program for radiation emergency medicine and construct a network of regenerative medicine for radiation emergency medicine. The establishment of new therapies by comprehensively using cell therapy, cellular repair and regenerative biotechnology will be promoted through collaboration with medical experts in various fields, including those involved in basic research as well as graduate...
In this review, we focus on the establishment of a system of regenerative medicine for radiation emergencies.

**Fig. 1. Development of a regenerative medical system for radiation emergencies.**

students, postdoctoral researchers, research coordinators, doctors and nurses. In cooperation with the Radiation Emergency Medical Preparedness and Assistance Network of World Health Organization (WHO-REMPAN), workers in the research center are developing a world-class center for radiation emergency medicine.

In this review, we focus on the establishment of a system of regenerative medicine for radiation emergencies.

**IMPROVEMENT OF EXISTING THERAPIES AND DEVELOPMENT OF NEW THERAPIES USING CELL THERAPY, CELLULAR REPAIR AND REGENERATIVE BIOTECHNOLOGY**

First, we should promote collaborative research for the improvement of existing therapies and development of new therapies using cell therapy, cellular repair and regenerative biotechnology that can be applied to regenerative medicine for radiation emergencies (Fig. 1).

Acute radiation syndrome is an acute illness caused by exposure of the whole body to ionizing radiation over a short period of time and has four distinct stages: prodromal stage, latent stage, illness stage and recovery or death [7-9]. Depending on the radiation exposure dose, hematopoietic (2–10 Gy), gastrointestinal (>10 Gy) and neurocardiovascular syndromes (>30 Gy) can occur [7-9].

On 30 September 1999, a nuclear criticality accident occurred at a uranium conversion fuel processing facility of JCO Co. Ltd. in Tokaimura, Japan. Three workers were exposed to heavy doses of neutrons and gamma-rays. One worker was exposed to 5.4 Gy of neutrons and 8.5 Gy of gamma-rays, and another worker was exposed to 2.9 Gy of neutrons and 4.5 Gy of gamma-rays. Those two workers died 82 days and 210 days after the criticality accident [1, 2]. One of the three workers was exposed to 0.81 Gy of neutrons and 1.3 Gy of gamma-rays and recovered after receiving multimodal therapy including treatment with granulocyte colony-stimulating factor [1, 2]. On 2 September 1982, in Kjeller, Norway, a worker in a radiation processing plant was exposed to ∼23 Gy of gamma-rays and died of acute radiation syndrome 13 day after exposure [10]. Treatment of advanced acute radiation syndrome remains very difficult, and some cases have no optional symptomatic treatment. The workers exposed to heavy doses of radiation died of acute radiation syndrome and continuous multiple organ damage despite the fact that bone marrow cell transplantation was successfully performed, suggesting that people with exposure of the whole body to heavy doses of radiation cannot survive even with successful bone marrow cell transplantation. Cell therapy using stem cells including MSCs and ADRCs is a promising strategy for treating acute radiation syndrome. However, if the whole body has been exposed to heavy doses of radiation, cell therapy will not be effective for acute radiation syndrome. Cell therapy would enable repair of localized radiation-induced injuries including injuries to the skin, soft tissue, skeletal muscle and nerves. Guidelines for the management and treatment of acute radiation syndrome are required.

Cell therapy for angiogenesis using bone-marrow mononuclear cells (BM-MNCs) in patients with peripheral arterial disease (PAD) was the first successful trial for human patients [11]. In 1997, Asahara et al. demonstrated for the first time that circulating EPCs may contribute to re-endothelialization of the injured endothelium and neovascularization at sites of ischemia [12]. EPCs are derived from bone marrow and have surface markers such as CD34+/AC133+/Tie2. Putative
Fig. 2. Methods for autologous BM-MNC implantation: bone-marrow puncture under general anesthesia, aspiration of bone marrow from the ileum, isolation of BM-MNCs using a cell separator, isolation of BM-MNCs using a blood cell separator to obtain a final volume, and implantation of BM-MNCs.

EPCs or angioblasts were isolated from human peripheral blood by magnetic bead selection on the basis of cell surface antigen expression. These cells differentiated into vascular endothelial cells. In animal models of ischemia, heterologous, homologous and autologous EPCs were incorporated into sites of active angiogenesis. These findings suggest that EPCs may be useful for augmenting growth of collateral vessels to ischemic tissues as so-called therapeutic angiogenesis and for delivering antiangiogenic or proangiogenic agents to sites of pathologic angiogenesis. Shintani et al. reported that the use of BM-MNCs, including EPCs, was sufficient and effective for therapeutic angiogenesis in rabbit models of limb ischemia [13]. Numerous collateral vessels developed in BM-MNC-transplanted rabbits but not in control or BM-fibroblast-transplanted animals. Quantitative analyses using an angiographic score showed a significantly greater number of collateral vessels in the BM-MNC group than in the other two groups in ischemic tissues. Immunohistochemical staining for von Willebrand factor and for alkaline phosphatase revealed the presence of numerous capillary endothelial cells in the BM-MNC-transplanted rabbits, but a smaller number of capillary endothelial cells was seen in the control and BM-fibroblast-transplanted animals. Quantitative analyses revealed that the capillary density in the ischemic region was significantly higher in the BM-MNC group than in the other two groups. The capillary:muscle fiber ratio was also greater in the BM-MNC group than in the other two groups. A greater degree of blood perfusion was observed in the ischemic limb of the BM-MNC-transplanted rabbits than in control and BM-fibroblast-transplanted animals. Although marked recovery of blood perfusion was observed in the BM-MNC-transplanted group, blood flow remained poor in the other two groups. The present study has several important clinical implications. First, autologous transplantation of BM-MNCs may represent a new and promising strategy for clinical application designed to revascularize ischemic tissues. Second, the fact that transplanted BM-MNCs participate in active angiogenesis in adult tissues suggests a potential utility of BM-MNCs as vectors for gene delivery to angiogenic sites in vivo.

Unfortunately, ~1–2% of patients with PAD progress to the stage of critical limb ischemia (CLI) and have to undergo major amputation [14]. Recently, angiogenic cell therapy or gene therapy has been attempted in these patients, and the feasibility of such therapy was shown [10,15–19]. Since 2002, we have also been performing autologous BM-MNC implantation in patients with CLI who have no optional therapy. As shown in Fig. 2, methods for autologous BM-MNC implantation include (i) bone-marrow puncture under general anesthesia, (ii) aspiration of about 500 mL of bone marrow from the ileum of each patient, (iii) immediate isolation of BM-MNCs using a CS3000-Plus blood-cell separator (Baxter, Deerfield, IL, USA), (iv) isolation of BM-MNCs using the blood cell separator CS3000-PLUS to obtain a final volume of ~50 mL, and (v) implantation of ~1 mL of isolated BM-MNCs (total cell number 1 x 10^8) into each injection site (total of 40–100 sites, 1.5 cm in depth) in the gastrocnemius of each ischemic leg with a 3 x 3 cm grid using a 22-gauge needle.

Several investigators including us have clearly shown that cell therapy is safe, feasible and effective, and it has been established since the first trial of autologous BM-MNC implantation in CLI patients who had no optional therapy in 2002 [10,15–19]. In order to confirm the long-term effect of BM-MNC implantation on clinical outcomes, we assessed the rates of major amputation and mortality after BM-MNC
implantation in 50 patients with critical limb ischemia, including 24 patients with PAD and 26 patients with Buerger’s disease [20]. The estimated 4-year amputation-free rates were 50% in patients with PAD and 4% in patients with Buerger’s disease. The amputation-free rate was markedly worse in patients with PAD than in patients with Buerger’s disease. The 4-year amputation-free rates after BM-MNC implantation were 100% in control patients with atherosclerotic PAD and 94% in control patients with Buerger’s disease.

In addition, we confirmed in vitro differentiation of attached cells derived from BM-MNCs into osteocytes [21]. Despite fasciotomy, many arteries collapsed and bone union in tibia and fibula fractures did not occur in a patient with compartment syndrome after a traffic accident who had tibia and fibula bone fractures, collapse of arteries and severe swelling of muscles. Autologous BM-MNC implantation for therapeutic angiogenesis and subsequent bone regeneration was performed in the injured leg. Four weeks later, angiography showed a marked increase in collateral vessels around the tibia fracture, and union of the fractured bone was completed 6 months later. BM-MNC implantation therapy may provide a new strategy for therapeutic osteogenesis.

Some mechanisms of cell therapy in angiogenesis have been postulated (Fig. 3) [22]. PAD is associated with endothelial dysfunction. Therefore, it is clinically important to evaluate the vascular function of collateral arteries induced by BM-MNC implantation. BM-MNC implantation improves not only limb ischemic symptoms and findings of angiography but also endothelial function in patients with CLI. Although the mechanism by which BM-MNC implantation improves endothelial function in patients with PAD is not clear, both differentiation of implanted cells per se and cell mobilization by angiogenic growth factors should contribute to cell therapy-induced augmentation of endothelial function. Nitric oxide (NO) is essential for re-endothelialization [23]. Vascular-induced mobilization of EPCs was lacking in endothelial NO synthase (eNOS)-knockout mice [24]. Overexpression of eNOS promoted effective angiogenesis through an increase in vascular endothelial growth factor (VEGF) expression in a rat model of hindlimb ischemia [25]. Therefore, therapeutic angiogenesis increases NO production, and then increased NO restores VEGF-induced mobilization of EPCs, leading to effective angiogenesis in patients with PAD. A decrease in NO inactivation caused by oxidative stress may also contribute to the improvement in endothelial dysfunction in patients with PAD. Impaired NO bioavailability may contribute to reduction in neovascularization in response to various stimuli, including angiogenic growth factors, aerobic exercise and pharmacological therapy, in patients with PAD. In addition, we emphasize that implanted cells do not differentiate into endothelial cells. Cell therapy induces mobilization of EPCs from bone marrow and is equivalent to gene therapy.

Angiogenesis is promising using any cells, BM cells, BM-MNCs, peripheral mononuclear cells, hemangiopoetic stem cells, MSCs, ADRCs and iPS cells. iPS cells have pluripotency and differentiate into osteoblasts, chondrocytes, adipocytes, neurons, skeletal muscle cells, endothelial cells and vascular smooth muscle cells (Fig. 4). In addition, it is expected that the use of iPS cells will play a central role in the establishment of a cell bank system for regenerative medicine at the cell processing center. Various angiogenic approaches, including cell therapy and gene therapy, have been tried for revascularization of ischemic tissues in animal models of ischemia, and BM-MNC implantation has been shown to induce therapeutic angiogenesis in both ischemic limb models and patients with limb ischemia [10, 15–19, 26, 27]. However, BM-MNC implantation requires harvesting a
large amount of BM under general anesthesia, which would be a burden for patients with severe complications such as myocardial ischemia, heart failure, cerebrovascular disease and renal failure.

Recently, we have focused on the potential of MSCs. MSCs can be easily isolated from BM and can be rapidly expanded \textit{ex vivo} [28]. Autologous MSCs have advantages over BM-MNCs and embryonic stem cells. When MSCs are used, the amount of aspirated BM can be markedly reduced, cell implantation can be repeatedly performed, there is no formation of carcinoma such as teratocarcinoma and hemangiosarcoma, there is no immune rejection and there are no ethical problems. Human MSCs have pluripotency and differentiate into osteoblasts, foam cells, chondrocytes and adipocytes (Fig. 5, D–F) [29, 30]. It is well known that human MSCs secrete growth factors and cytokines including VEGF, fibroblast growth factor, epidermal growth factor, tumor necrosis factor alpha and interleukins 6, 8 and 11 [31, 32]. It is thought that angiogenic cytokines secreted from MSCs induce increases in proliferation and mobilization of endothelial cells. However, it is controversial whether MSCs directly differentiate into endothelial cells \textit{in vitro} [33–35]. In a culture medium, MSCs formed a monolayer of adherent cells and resembled long spindle-shaped fibroblastic cells. Adherent spindle-shaped cells were seen in the culture dish at \( \sim 4–5 \) days after initial plating and rapidly reached semiconfluence in the culture medium at \( \sim 8–12 \) days after initial plating (Fig. 5, A and B). MSCs do not have a specific antigen profile. However, we confirmed by using real-time PCR that MSCs were negative for typical hematopoietic antigens CD34, CD38 and CD45 and were positive for molecular markers (e.g. BMP4, IGF1, LIF and PRG1) [36, 37]. The MSC population expanded from \( 10^3 \) cells to \( 10^6 \) cells by 4–5 passages. The cumulative number of MSCs increased linearly from \( 5.1 \times 10^8 \pm 3.2 \times 10^8 \) to \( 3.0 \times 10^{12} \pm 1.8 \times 10^{12} \) at day 35 (Fig. 5, C) [28]. In a rabbit hind limb ischemic model, a large number of capillaries was detected in ischemic muscle of the MSC implantation group compared with that in the control group at day 28 after MSC implantation or saline injection [27]. Both capillary density score and capillary/muscle fiber ratio of the ischemic hindlimb were significantly higher in the MSC group than in the control group. Quantitative analysis of laser Doppler perfusion images (LDPIs) revealed an increase in blood flow after implantation of MSCs in ischemic hindlimbs in comparison with blood flow in control hindlimbs that received injection of saline. Time-dependent change in the LDPI index after implantation of MSCs or saline injection was significantly greater in the MSC group than in the control group. Marked formation of new collateral vessels in the lower limbs was seen at day 28 after MSC implantation, whereas angiography showed poor collateral vessel formation in lower limbs of the control group. The angiographic score at day 28 after MSC implantation was significantly higher in the MSC group than in the control group. The response of leg blood flow to infusion of acetylcholine was greater in the MSC group than in the control group at the end of the 28-day study period. In a very small number of fields in ischemic limb tissues (1 per
Fig. 5. (A) At day 5 after initial plating, adherent cells were spindle-shaped. (B) At day 10 after initial plating, the cells had grown to semiconfluence (original magnification: ×100). (C) Line graph shows the relationship between cumulative number of cells and number of days after MSC plating. (D) Osteogenic differentiation, anti-osteocalcin. (E) Adipogenic differentiation, anti-fatty acid binding protein 4. (F) Cartilage differentiation, anti-aggrecan (modified from refs. 25–27).

∼2000 slices of samples of the adductor muscle and semimembranosus muscle), enhanced green fluorescent protein (EGFR)-positive and CD31/EGFP merged cells were detected in the endothelial capillary structure at day 28 after MSC implantation. These merged cells were present near and within necrotic or scar lesions in ischemic limb tissues. EGFP-positive and CD31/EGFP merged cells were not detected in ischemic limb tissues, while CD31-positive cells were detected in the endothelial capillary structure in all rabbits that had effective angiogenesis by MSC implantation. A large part of MSC implantation-induced angiogenesis should be due to mobilization of endothelial progenitor cells by angiogenic growth factors. It is likely that a part of the transdifferentiation of MSCs into endothelial cells is involved in angiogenesis in severely injured areas, although the degree of contribution to angiogenesis with transdifferentiation of MSCs is very low.

Autologous MSCs have several advantages over BM-MNCs and embryonic stem cells: (i) the amount of aspirated or collection of BM can be markedly reduced because the number of MSCs can be rapidly increased (in regimes of BM-MNC implantation, ∼500–700 mL of BM is aspirated from the ileum under general anesthesia), (ii) MSCs can be banked after cell culture, and cell implantation can be repeatedly performed, and (iii) there are no ethical problems. In addition, several studies have shown that MSCs per se have immunoregulatory properties. Allogenic MSCs have recently been shown to suppress T cell proliferation in both in vitro and in vivo studies [38]. Indeed, administration of MSCs safely allowed transplantation of donor cells into conditioned recipients without the use of pharmacologic immunosuppression [39]. Co-infusion of allogenic human MSCs and donor cells can prevent or be used for treatment of acute-graft-versus-host disease in patients who have undergone BM transplantation [40]. These findings indicate the possibility of using allogenic MSC implantation for angiogenesis.

Recently, we have also focused on ADRCs. We present a few ideas concerning the possibility of using ADRCs (Fig. 6). ADRCs, sometimes referred to as stromal vascular fraction cells, are a heterogeneous or mixed population of cells found in adipose tissue [41, 42]. This population includes adult stem cells, EPCs, leukocytes, endothelial cells and vascular smooth muscle cells. Autologous adult stem and regenerative cells are thought to promote healing of scarred or injured tissue. While we are learning more about the exact mechanisms every day, it is believed that this heterogeneous population of cells influences the local environment via cell-to-cell signaling, immune modulation and differentiation into other cell types. The use of ADRCs for treatment of many different medical conditions, including cardiovascular disease, soft tissue defects and wound healing, is being evaluated in numerous clinical and preclinical studies around the world. The use of ADRCs is a unique and promising approach and has key advantages over stem and regenerative cells from other sources. While stem and progenitor cells usually make up <5% of all ADRCs, this percentage is 2500-fold larger than the percentage of such cells in tissues such as bone marrow (0.002%). The abundance of ADRCs in adipose tissue and the ability to easily collect large amounts of adipose tissue via liposuction eliminates the need for tissue culturing. It is thought that autologous cells cannot be used in most cases of whole body exposure to strong radiation. However, it is expected that autologous cells can be used in some cases. If so, this system would be very useful to obtain an adequate
amount of cells for implantation in the acute phase after radiation-induced injury.

Recently, cell therapy has been used for angiogenesis, regeneration of cartilage and anti-cancer recurrence and has been shown to be safe, feasible and effective [10, 15–19, 21]. However, regeneration of the digestive tract using cell therapy has not been established. Uncontrolled digestive tract hemorrhage was a critical problem after severe radiation exposure in the Tokai-Mura accident. If we cannot use autologous cells for regenerative medicine when encountering a radiation disaster, the use of allogenic cells should be considered. In such cases, it would be necessary to stock allogenic cells as well as autologous cells in the cell bank. The preparation of a cell bank system is essential for the establishment of regenerative medicine for radiation emergencies. In addition, there are still many problems to be solved for cell therapy after severe radiation exposure other than regeneration of the digestive tract.

DEVELOPMENT OF HUMAN RESOURCES
It is important to develop human resources for radiation emergency medicine, especially regenerative medicine (Fig. 1). The development of human resources including training and education for nuclear disasters is continuing in industry, regulatory agencies, universities, research institutes, local communities and countries with the cooperation of the IAEA and international cooperation. However, in addition to the small number of people who are involved in the nuclear safety field, it is difficult to obtain adequate professional human resources for radiation emergency medicine. The establishment of human resources is time-consuming. In Hiroshima University, Phoenix Leader Education Program for Renaissance from Radiation Disasters was adopted as one of the leading PhD programs that are projects of the Ministry of Education, Science and Technology in Japan. These programs will lead to recovery from radiation disasters and establishment of a new social system that is safe and secure and serves as a model for human society in the 21st century. It is necessary to implement a permanent network of industry, academia and governments that is able to support the development of human resources both academically and practically.

ESTABLISHMENT OF A NETWORK
Finally, there is an urgent need for the establishment of networks of regenerative medicine for radiation emergencies, including both domestic and international networks (Fig. 1). In 1987, WHO-REMPAN was established to provide technical assistance, education, training, research coordination and sharing of information for radiation emergencies. Recently, the concept of WHO-REMPAN has been spreading worldwide and has been contributing to the responses to radiation emergency accidents. Unfortunately, at present, WHO-REMPAN does not explicitly refer to the necessity of regenerative medicine for radiation emergency medicine. However, we have already experienced criticality accidents that have occurred at nuclear power plants around the world. The possibility of future disasters from nuclear plant accidents and nuclear terrorism cannot be ruled out. We must also consider radiation exposure induced by the sources of radioactive devices in a medical setting. It is necessary to establish a global network for regenerative medicine for radiation emergencies.

CONCLUSION
With a view to developing an efficient cell therapy system for radiation emergency medicine that would effectively facilitate and help to enhance tissue regeneration, after the Fukushima nuclear plant accident in 2011 we took the first step toward the establishment of regenerative medicine for radiation emergencies.

SUPPLEMENT FUNDING
This supplement has been funded by the Program of the Network-type Joint Usage/Research Center for Radiation Disaster Medical Science of Hiroshima University, Nagasaki University, and Fukushima Medical University.

ACKNOWLEDGMENTS
We thank Megumi Wakisaka, Ki-ichiro Kawano and Satoko Michiyama for their excellent secretarial assistance. This work was presented at the Fourth International Symposium of the Joint Usage/Research Center held on 12 February 2020 Hiroshima.
REFERENCES

1. Hirama T, Tanosaki S, Kandatsu S et al. Initial medical management of patients severely irradiated in the Tokai-muracruciality accident. Br J Radiol 2003;76:246–53.

2. Sumption N, Ainsbury L, Goodhead D et al. High frequency of simple and complex chromosome aberrations detected in the Tokai-mura survivor four and five years after the 1999 criticality accident. J Radiat Res 2011;52:300–8.

3. Durdu S, Akar AR, Arat M et al. Autologous bone-marrow mononuclear cell implantation for patients with Rutherford grade II-III thromboangiitis obliterans. J Vasc Surg 2006;44: 732–9.

4. Higashi Y, Kimura M, Harasaka et al. Autologous bone-marrow mononuclear cell implantation improves endothelium-dependent vasodilation in patients with limb ischemia. Circulation 2004;109:1215–8.

5. Lederman RJ, Mendelsohn FO, Anderson RD et al. TRAFFIC investigators. Therapeutic angiogenesis with recombinant fibroblast growth factor-2 for intermittent claudication (the TRAFFIC study): A randomised trial. Lancet 2002;359:2053–8.

6. Rajagopal S, Mohler ER 3rd, Lederman RJ et al. Regional angiogenesis with vascular endothelial growth factor in peripheral arterial disease: A phase II randomized, double-blind, controlled study of adenosine delivery of vascular endothelial growth factor 121 in patients with disabling intermittent claudication. Circulation 2003;108:1933–8.

7. Donnelly EH, Nemhauser JB, Smith JM et al. Acute radiation syndrome: Assessment and management. South Med J 2010;103:541–6.

8. Dörr H, Meineke V. Acute radiation syndrome caused by accidental radiation exposure - therapeutic principles. BMC Med 2011;9:126.

9. Acosta R, Warrington SJ. Radiation Syndrome. 2020 Jul 21StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing, 2020.

10. Stavem P, Bregger A, Devik F et al. Lethal acute gamma radiation accident at Kjeller, Norway. Report of a case. Acta Radiol Oncol 1985;24:61–3.

11. Tateishi-Yuyama E, Matsubara H, Murohara T et al. Therapeutic angiogenesis for patients with limb ischaemia by autologous transplantation of bone-marrow cells: A pilot study and a randomized controlled trial. Lancet 2002;360:427–35.

12. Asahara T, Murohara T, Sullivan A et al. Isolation of putative progenitor endothelial cells for angiogenesis. Science 1997;275:964–7.

13. Shintani S, Murohara T, Ikeda H et al. Augmentation of postnatal neovascularization with autologous bone marrow transplantation. Circulation 2001;103:897–903.

14. Norgren L, Hiatt WR, Dormandy JA et al. TASC II working group. Inter-society consensus for the Management of Peripheral Arterial Disease (TASC II). J Vasc Surg 2007;45:S5–67.

15. Motukuru V, Suresh KR, Vivekanand V et al. Therapeutic angiogenesis in Buerger’s disease (thromboangiitis obliterans) patients with critical limb ischaemia by autologous transplantation of bone marrow mononuclear cells. J Vasc Surg 2008;48:535–605.

16. Miyamoto K, Nishigami K, Nagaya N et al. Unblinded pilot study of autologous transplantation of bone marrow mononuclear cells in patients with thromboangiitis obliterans. Circulation 2006;114:2679–84.

17. Huang P, Li S, Han M et al. Autologous transplantation of granulocyte colony-stimulating factor-mobilized peripheral blood mononuclear cells improves critical limb ischemia in diabetes. Diabetes Care 2005;28:2155–60.

18. Kalka C, Masuda H, Takahashi T et al. Vascular endothelial growth factor (165) gene transfer augments circulating endothelial progenitor cells in human subjects. Circ Res 2000;86:1198–202.

19. Yusoff FM, Kajikawa M, Matsu S et al. Review of the long-term effects of autologous bone-marrow mononuclear cell implantation on clinical outcomes in patients with critical limb ischemia. Sci Rep 2019;9:7711.

20. Idei N, Chowdhury M, Soga J et al. Autologous bone-marrow mononuclear cell implantation reduces long-term major amputation risk in patients with critical limb ischemia: A comparison of atherosclerotic peripheral arterial disease and Buerger disease. Circ Cardiovasc Inter 2011;4:15–25.

21. Umemura T, Nishioka K, Ochi M et al. Autologous bone-marrow mononuclear cell implantation induces angiogenesis and bone regeneration in a patient with compartment syndrome. Circ J 2006;70:1362–4.

22. Higashi Y, Nishio K, Umenura et al. Oxidative stress, endothelial function, and angiogenesis induced by cell therapy and gene therapy. Curr Pharm Biotechnol 2006;7:109–16.

23. Murohara T, Asahara T, Silver M et al. Nitric oxide synthase modulates angiogenesis in response to tissue ischemia. J Clin Invest 1998;101:2567–78.

24. Aicher A, Heeschen C, Mildner-Rihm C et al. Essential role of endothelial nitric oxide synthase for mobilization of stem and progenitor cells. Nat Med 2003;9:1370–7.

25. Namba T, Koike H, Murakami K et al. Angiogenesis induced by endothelial nitric oxide synthase gene through vascular endothelial growth factor expression in a rat hindlimb ischemia model. Circulation 2003;108:2250–7.

26. O’Neill TJ 4th, Wamhoff BR, Owens GK et al. Mobilization of bone marrow-derived cells enhances the angiogenic response to hypoxia without transdifferentiation into endothelial cells. Circ Res 2005;97:1027–35.

27. Silva GV, Litovsky S, Assad JA et al. Mesenchymal stem cells differentiate into an endothelial phenotype, enhance vascular density, and improve heart function in a canine chronic ischemia model. Circulation 2005;111:150–6.

28. Mikami S, Nakashima A, Nakagawa K et al. Autologous bone-marrow mesenchymal stem cell implantation and endothelial function in a rabbit ischemic limb model. PLoS One 2013;8:e67739.

29. Takigawa H, Kitadai Y, Shinagawa K et al. Multikinase inhibitor regorafenib inhibits the growth and metastasis of
colon cancer having abundant stroma. *Cancer Sci* 2016;107:601–8.

30. Takigawa H, Kitadai Y, Shinagawa K et al. Mesenchymal stem cells induce epithelial to mesenchymal transition in colon cancer cells through direct cell-to-cell contact. *Neoplasia* 2017;19:429–38.

31. Kilroy GE, Foster SJ, Wu X et al. Cytokine profile of human adipose-derived stem cells: Expression of angiogenic, hematopoietic, and pro-inflammatory factors. *J Cell Physiol* 2007;212:702–9.

32. Strioga M, Viswanathan S, Darinskas A et al. Same or not the same? Comparison of adipose tissue-derived versus bone marrow-derived mesenchymal stem and stromal cells. *Stem Cells Dev* 2012;21:2724–52.

33. Jazayeri M, Allameh A, Soleimani M et al. Molecular and ultrastructural characterization of endothelial cells differentiated from human bone marrow mesenchymal stem cells. *Cell Biol Int* 2008;32:1183–92.

34. Roobrouck VD, Clavel C, Jacobs SA et al. Differentiation potential of human postnatal mesenchymal stem cells, mesoangioblasts, and multipotent adult progenitor cells reflected in their transcriptome and partially influenced by the culture conditions. *Stem Cells* 2011;29:871–82.

35. Bronckaers A, Hilkens P, Martens W et al. Mesenchymal stem/stromal cells as a pharmacological and therapeutic approach to accelerate angiogenesis. *Pharmacol Ther* 2014;143:181–96.

36. Kanawa M, Igarashi A, Fujimoto K et al. Genetic marker can predict chondrogenic differentiation potential in bone-marrow derived mesenchymal stromal cells. *Stem Cells Int* 2018;2018:9530932.

37. Kinnaird T, Stabile E, Burnett MS et al. Marrow-derived stromal cells express genes encoding a broad spectrum of arteriogenic cytokines and promote in vitro and in vivo arteriogenesis through paracrine mechanisms. *Circ Res* 2004;94:678–85.

38. Tse WT, Pendleton JD, Beyer WM et al. Suppression of allogeneic T-cell proliferation by human marrow stromal cells: Implications in transplantation. *Transplantation* 2003;75:389–97.

39. Beggs KJ, Lyubimov A, Borneman JN et al. Immunologic consequences of multiple, high-dose administration of allogeneic mesenchymal stem cells to baboons. *Cell Transplant* 2006;15:711–21.

40. Le Blanc K, Rasmusson I, Sundberg B et al. Treatment of severe acute graft-versus-host disease with third party haploidentical mesenchymal stem cells. *Lancet* 2004;363:1439–41.

41. Gimble JM, Katz AJ, Bunnell BA et al. Adipose-derived stem cells for regenerative medicine. *Circ Res* 2007;100:1249–60.

42. Bunnell BA, Flaat M, Gagliardi C et al. Adipose-derived stem cells: Isolation, expansion and differentiation. *Methods* 2008;45:115–20.