Cell Therapy for Cardiovascular Diseases
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There have been great progresses in our knowledge of patho-physiology on various cardiovascular diseases, which enabled us to develop the field of regenerative medicine for previously untreated patients. Among several strategies in cardiovascular regenerative medicine, cell transplantation is one of the best studied and the best clinically practiced. In this review we will first summarize the mechanisms of cell therapy, and then go through lists of cells and diseases that can be applied. Later we will introduce some of the clinical experiences published so far, with some discussion regarding the problems and perspectives of this novel therapeutics.

Key words: neovascularization, angiogenesis, peripheral artery disease, ischemic heart disease

INTRODUCTION

Cardiovascular diseases are the leading cause of morbidity and mortality in the industrialized communities, despite the considerable evolution of medicine in this field. Contrary to high hopes for regenerative medicine as a breakthrough to these problems, our knowledge and techniques are quite limited to meet those anticipations. Nevertheless there have been some progresses both in basic and clinical science, which will be introduced in this brief review. We will start by summarizing the mechanisms of cell therapy, followed by going through the lists of cells and diseases that can be applied. Later we will introduce some of the clinical experiences published so far, with some discussion regarding the problems and perspectives of this novel therapeutics.

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MECHANISM OF CELL THERAPY FOR CARDIOVASCULAR DISEASES

The cardiovascular system is composed of not only the cells that constitute heart and vessels, but also of humoral and cellular factors that control homeostasis and pathology of these cells. Elucidation of such factors bargains us to realize the application of cell therapy in various cardiovascular diseases. In the beginning, we will introduce these putative mechanisms how cell transplantation can mend these broken hearts and vessels.

Replenishment of Vascular Components

Blood vessels constitute the core component of the cardiovascular system. Most of the cardiovascular diseases imply damage of blood vessels ranging from endothelial dysfunction to violent destruction of the total vessel structure. Recent progress in cellular and molecular biology revealed some compensatory mechanisms essentially underlying these events.

Among those, reconstruction of lost blood vessels following tissue ischemia, termed neovascularization, is extensively studied in the past two decades. There are three major mechanisms known in neovascularization. Angiogenesis, sprouting of endothelial cells from existing ones, requires endothelial cells proliferation, migration, and survival. Post natal vasculogenesis involves endothe-
lial progenitor cells (EPCs), which resides in the bone marrow while quiescent, and then mobilized into peripheral circulation and recruited to the site of vessel construction, where they terminally differentiate into mature endothelial cells. A mechanism which forms collateral vessels bypassing an occluded conducting artery is called arteriogenesis. In arteriogenesis, monocytes and macrophages play crucial roles by interacting with endothelial cells. These knowledge indicates that administration of EPCs and other stem/progenitor cells that have a potential to differentiate into vascular components may induce post natal vasculogenesis in ischemic foci.

Endothelial damage will be another target for cell therapy. Angioplasty related complication is a major unsolved problem in the current cardiovascular medicine. Post angioplasty restenosis and thrombosis is known to be a consequence of endothelial denudation. Endothelium covers the internal surface of the vasculature with only a single-cell lining, and is easily detached from the vascular wall by mechanical harassment with balloons and stents. This results in abrupt thrombosis, followed by inflammation that induces matrix deposition and cellular proliferation to finally form post angioplasty restenosis. In order to prevent these adverse consequences, damaged endothelial lining should quickly be replaced. Use of stents that gradually elute anti-mitotic drug (drug eluting stent: DES) may be favorable in preventing neointima formation, but not in the occurrence of late thrombosis. Supplement of endothelium to the site of these interventions may prevail against such complications. This could also be applied for the production of valvular or vascular prostheses resistant to thrombosis.

Replenishment of Tissue Component Cells

Heart failure is a progressive disease that involves cardiomyocyte hypertrophy and death. Lost cardiomyocytes are replaced by fibrosis that results in marked reduction of myocardial function. This owes to the fact that cardiomyocytes do not replicate postnatally. Thus, effective replenishment of cardiomyocytes would be an ideal strategy to treat patients with this previously irreversible disease. One of the strong candidates for the extrinsic source of cardiomyocytes is the embryonic stem (ES) cells and induced pluripotent stem (iPS) cells. These cells were shown to differentiate into cardiomyocytes in vitro, and to form functional myocardium when transplanted experimentally in vivo. However there still lie some major difficulties for the cells to be applied for any clinical settings, as will be discussed in the later section. Another source of extrinsic cardiac stem cells can be found within the bone marrow. In the early 2000’s, several reports showed that bone marrow derived hematopoietic and stromal cells can differentiate into cardiomyocytes in adult heart.21 There were also reports showing that such
cells can regenerate considerable amount of de novo myocardium when transplanted into the infarct heart. However, this was challenged by recent studies showing that bone marrow derived hematopoietic cells seldom, if ever, transdifferentiate into cardiomyocytes. The mismatch between these conclusions might have emerged from technical problems, which give a decision in favor of denying the capacity of bone marrow hematopoietic cells to regenerate myocardium. The ability of bone marrow stromal cells to differentiate into cardiomyocyte has not been denied, but the general consensus is that the event is so rare to induce functional benefit, indicating the involvement of other mechanisms besides cardiomyogenesis.

**Tissue Protection and Regeneration by Paracrine effect**

Neovascularization in ischemic foci requires various types of cells including endothelial cells, vascular smooth muscle cells, fibroblasts, macrophages, and EPCs. The behaviors of those cells are governed by various angiogenic growth factors, cytokines and chemokines. Because bone marrow and peripheral blood hematopoietic cells can be sources of such factors, cell transplantation could contribute much to neovascularization by their secretion. This is termed the paracrine effect. Paracrine effect is coming to be recognized as the major mechanism of cell therapies. Recent studies demonstrated that even stem cells have tremendous ability to secrete growth factors when implanted into ischemic tissue, and that the therapeutic effect of these stem cells paradoxically relies on the paracrine effect. The factors produced by these cells contribute not only to neovascularization, but also to the protection of tissue facing severe insults, by means of inhibiting excessive apoptosis of the tissue component cells facing disease insults.

However, it is also becoming increasingly recognized that paracrine effect by transplanted cells itself do not last long enough to induce neovascularization nor tissue protection, for most of the cells are shown to be quickly lost from the site of their injection. Investigations on skeletal muscle and myocardial ischemia showed that production of growth factors prolongs for a long period of time, and that the source of these soluble factors was the host muscle tissue that is undergoing its own regeneration processes. This account for the third target of the paracrine effect in cell therapies, and is more likely to be the main mechanism of the therapy.

**In Situ Activation of Resident Stem Cells**

There has been reports indicating that cell transplantation induce in situ activation of resident stem cells. In ischemic skeletal muscle, administration of peripheral blood mononuclear cells induced activation and marked proliferation of the skeletal muscle specific progenitor cells, resulting in coordinated muscle regeneration and enhanced neovascularization. This indicates the fourth major mechanism how cell transplantation induce tissue regeneration.

This seems to be true in the myocardium as well. There are reports proving the intrinsic pool of cardiac progenitor cells. These cells reside quiescent in the myocardium and are thought to be responsible for replacement of the parenchymal cells lost by normal wear and tear. However, in response to stress, these progenitors go into self-sustaining activation and form a paracrine loop between these progenitors and other muscle cells. In a porcine model of myocardial infarction, allogeneic mesenchymal stem cells (MSCs) stimulated myocardial progenitors to enter cell cycle, suggesting that effective cell therapy could lead to restoration of stem cell niches through multifaceted cell–cell interactions. In both cases, extent of cardiomyogenesis was quite low and could not account for the functional benefit of cell infusion. However, the results from skeletal and cardiac muscle strongly suggest the possibility that activation of tissue resident stem and progenitor cells should govern tissue regeneration in a coordinated manner.

Recently, we have found a factor that effectively induces differentiation of cardiomyocyte by inhibiting the canonical Wnt signaling. If such factors could effectively be used in mammalian to activate and expand these cardiac progenitors, this sure will be another strategy to therapeutically replenish lost cardiomyocytes. Progress in this field is also awaited.

**Types of Cell to be used in Cardiovascular Diseases**

Given the machinery framework of cell therapy, we can now make selections of the concrete types of cells to be used. In this section we will introduce the types of cells that have been explored and have successfully been used in the basic investigations.

**Embryonic Stem Cells and Induced Pluripotent Stem Cells**

Since their establishment, both mouse and human ES cells have extensively been explored. ES cells are shown to differentiate into various components of myocardium
in vitro, including endothelial cells, vascular smooth muscle cells and cardiomyocytes. Moreover the ES derived progenitor cells could successfully be engrafted into experimentally damaged myocardium, contributing to its functional recovery by differentiating terminally into each tissue components. Recently, iPS cells from human fibroblasts have been established. IPS cells have similar capacities found in the ES cells, including differentiating into cardiomyocytes and other component of myocardium. It has already been shown that iPS derived cells could reconstitute cardiomyocytes and enhance functional recovery of the heart just as the ES cells did (Yamashita et al, personal communications). Moreover, iPS cells calls less for the ethical barriers clinical administration of ES cell owes. However, both ES and iPS are tempered by the high incidence of forming teratomas after transplantation, which makes these cells currently restricted from clinical application.

Mesenchymal Stem Cells (MSCs)

Mesenchymal stem cells (MSCs) are non-hematopoietic multi-potent stem-like cells that are capable of differentiating into multiple lineages in vitro and in vivo, including bone, cartilage, fat, neurons, astrocytes and myoblasts. One of the major benefits of MSCs is that they can easily be isolated from a small aspirate of bone marrow, and can rapidly be expanded. However, there are still some open questions about their origin, multipotentiality and anatomical localization. Nevertheless, MSCs could be a good candidate for the source of cells to treat various cardiovascular diseases.

It was shown in vitro that approximately 30% of the MSCs can differentiate into cardiomyocytes when treated with 5-azacytidine. These cells were successfully engrafted to porcine myocardial scar after infarction, forming islands of cardiac-like tissue. The MSCs transplantation also induced angiogenesis in company with the improvement in the regional and global contractile function and the prevention of ventricular remodeling. Similar results were obtained from porcine allograft MSCs transplanted into infarcted myocardium.

The mechanism how MSCs induced its therapeutic efficacy has been a matter of debate. Although bone marrow cells were found to have become cardiomyocytes, the event occurred at a very low frequency, that could not account for the functional recovery following cell transplantation. Moreover, transgenical experiment revealed that this was not due to differentiation of MSCs, but was a result of cell fusion process occurring between administered transplants and native cardiomyocytes. More recent study revealed that a substantial portion of the curative effects of MSCs is attributable to protection of ischemic myocardium by a paracrine effect, or its ability to activate intrinsic regeneration processes, instead of de novo regeneration by cardiomyogenic differentiation of the donor cells.

Endothelial Progenitor Cells (EPCs)

Although there had been a circumstantial evidence that bone marrow derived cells may reconstitute endothelial cells in vascular prostheses, the presence of EPCs in peripheral circulation had never been directly evident until the proof made by Asahara et al in 1997. They showed that human peripheral blood mononuclear cells (PB-MNCs) can acquire an endothelial cell-like phenotype in vitro, and incorporate into capillaries when transplanted into animals. These putative EPCs were typified by expression of CD34 and VEGF receptor-2 (VEGFR-2) initially, then specified by various other markers in later studies. EPCs is known to play very important roles in the physiologic, pathologic, and therapeutic neovascularization.

The usefulness of EPCs on the treatment of ischemia was shown by Kalka et al who created hind limb ischemia in athymic nude mice and infused the EPCs isolated from PB-MNCs of a human donor. Mice receiving human EPCs exhibited significant recovery of blood flow and capillary density, which was sufficient to increase the chances of successful limb salvage. Meanwhile, Kawamoto et al. worked on nude rats with myocardial ischemia. Ex vivo expanded human EPCs incorporated into the foci of myocardial neovascularization and had a favorable impact on the preservation of left ventricular function. The mechanism was then thought to rely on the post natal vasculogenesis induced by administered EPCs.

Utility of EPCs is not restricted to the treatment of ischemia. In animals, seeding of CD34-positive cells or BM cells enhanced vascular graft endothelialization. Mobilization and incorporation of BM-derived EPCs has been shown to contribute to reendothelialization at the sites of endothelial cell damage. These findings warrant the development of thrombosis resistant vascular and valvular prosthesis by either coating the surface with EPCs ex vivo, or by coating antibodies that efficiently recruit endogenous EPCs after in-body replacement. Similarly, anti-CD34 coated stents may efficiently capture circulating EPCs that will differentiate into mature...
endothelial cells, which might prevent restenosis and thrombosis.

However, there are some apprehensions for the use of EPCs in a clinical setting. First, the identity of EPCs is complicated by the lack of reliable cell surface markers. This is partially due to distinct EPCs fractions showing different phenotypes in the time course of ex vivo culture. Second, EPCs from patients exposed to coronary risk factors exhibit an impairment in proliferation and migration capacity, suggesting the limitation of its usage. Finally, recent basic studies question the frequency of EPCs to be incorporated into adult vasculature. A study of mice myocardial infarction model revealed that only 3.3% of injected bone marrow cells incorporated into the vasculature. Another group analyzed hind limb sections by laser scanning confocal microscopy and found that none of the bone marrow derived cells have ever been incorporated into growing vasculature, and argued that previous investigations failed to distinguish false-positive results based on the use of conventional light or fluorescence microscopy. Regardless of the uncertainty of its precise mechanism, however, EPCs did have therapeutic efficacy in numerous animal studies. This has led to clinical application of EPCs on treatment of several ischemic diseases, which will be discussed in this paper later.

**Bone Marrow and Peripheral Blood Mononuclear Cells (BM-MNCs, PB-MNCs)**

Since MSCs and EPCs originate from bone marrow in adults, implantation of bulk bone marrow should also induce functional recovery of tissue damaged by ischemia. The strategy is beneficial in that it requires no ex-vivo maneuvers other than gradient cell separation protocol, which has already been established in the field of therapeutics in hematological diseases.

Use of bone marrow mononuclear cells (BM-MNCs) was tested in a rat model of hind limb ischemia, and found to be effective in collateral vessel formation and recovery of blood perfusion. Shintani et al. showed that bone marrow mononuclear cells do contain functional EPCs and that transplantation of autologous BM-MNCs can augment angiogenesis and collateral vessel formation in a rabbit model of hindlimb ischemia.

Bone marrow derived cells also can restore function of damaged heart. Injection of autologus bone marrow cells into a rat ischemic heart model induced significant angiogenesis that was associated with elevation of inflammatory cytokines. Feasibility of bone marrow cells to treat ischemia was pre-clinically confirmed by a translational research using a swine model of acute myocardial infarction. Intramuscular implantation of autologous BM-MNCs into sites of acute and chronic myocardium resulted in improvement of cardiac function in company with higher regional blood flow and capillary densities. BM-MNCs consist of a heterogeneous cell population including hematopoietic, mesenchymal, and other progenitor cells, as well as mononuclear cells. Therefore the treatment was thought to work in several distinct mechanisms. Cell transplantation induced expression of various angiogenic factors found in BM-MNCs, suggesting that the ability of the bone marrow cells to secrete such factors promoted neovascularization. They also found that transplant derived cells were incorporated into newly formed vasculature, concluding that post natal vasculogenesis also accounted for its therapeutic efficacies.

Bone marrow stem cells can be mobilized into peripheral circulation by administration of granulocyte colony stimulating factor (G-CSF). Injection of G-CSF–mobilized human CD34 + stem cells into rat infarct hearts induced neovascularization. Interestingly, newly formed vessels were of both rat and human origin, suggesting that human angioblasts or other co-administered BM-derived cells function as a rich source of proangiogenic factors in addition to being a direct contributor to in situ vessel formation. Recent basic studies suggested that paracrine effect mainly accounts for the therapeutic efficacy of cell transplantation.

Because PB-MNCs can also be a source of various growth factors, cytokines, and chemokines, such naïve cells without stem cells can also be a candidate for the source of therapy. Studies demonstrated that PB-MNCs can augment perfusion of ischemic limbs and heart to the extent comparable to that obtained by BM-MNCs implantation. Because PB-MNCs can be harvested in a much less invasive fashion, this can be a strong alternative to severely ill patients for instance who cannot undergo general anesthesia to obtain bone marrow cells.

**Skeletal Myoblasts**

Skeletal muscle cells are intrinsically contractile cells. They derive from tissue specific precursors, known as satellite cells or skeletal myoblasts. Skeletal myoblasts can be an attractive source of cells to be used, for they are of autologous origin, have high proliferative potential, retains virtually no risk of tumorigenicity, and possess a high resistance to ischemia. In these regards skeletal myoblasts are thought to be a good candidate for replac-
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Transplantation of myoblasts improved cardiac function after coronary artery ligation\(^{45}\) of animal hearts. In these models, islands comprising elongated, striated cells that retained characteristics of both skeletal and cardiac cells were found. However these cells did not couple electronically with native cardiomyocytes. Thus these benefits are thought to be due to paracrine factors produced by engrafted skeletal muscle cells.\(^{46}\)

Clinical Trials

All these basic and translational studies warranted the clinical application of cell therapy. Here we will introduce foremost clinical trials published so far that have treated previously incurable cardiovascular diseases by administration of various types of cells (Table 1 and Table 2).

### Peripheral Artery Disease

Most of the peripheral artery disease has been successfully treated by general, pharmaceutical, and surgical therapy. However, in the chronic critical limb ischemia (CLI) with ischemic ulcers or intolerable rest pain, the effects of these conventional therapies are limited, urging most of these patients to undergo major limb amputation. Because CLI involves loss of efficient collateral arteries and capillary beds, the pathology could have implications for transplantation of cells that have the ability to form new blood vessels. Experimentally, various types of cells including EPCs, BM-MNCs, and PB-MNCs could successfully revascularize ischemic skeletal muscle cells and prevent limb amputation.

The first clinical trial of cell therapy was reported in 2002, which treated patients with CLI by intramuscular injections of BM-MNCs (TACT study).\(^{47}\) The study consisted of a pilot study recruiting 25 patients, and a double-blind randomized prospective study which enrolled 20 patients. BM-MNCs implantation induced augmentation of lower limb blood pressure index and of tissue oxygen pressure, in company with clinical improvements in rest pain and pain-free walking time. These effects were seen from as early as 4 weeks after implantation, and sustained for more than 24 weeks. There were no major adverse events with clear association to the procedure. Consequently, the trial successfully showed the safety and effectiveness of autologous BM-MNCs implantation for therapeutic angiogenesis.

Clinical application of BM-MNCs is partially limited,
| Study (Ref)        | Ref | Publication | Design  | (N) | Patients | Cell type          | Delivery method       | Outcome                        | Adverse Events |
|-------------------|-----|-------------|---------|-----|----------|--------------------|------------------------|-------------------------------|----------------|
| Strauer et al     | 53  | 2002        | Serial  | 10  | AMI      | BM-MNCs           | Intra-coronary          | Feasibility and safety       |                |
| TOPCARE-AMI       | 54, 55 | 2002, 2004  | RCT     | 59  | AMI      | BM-MNCs or EPCs   | Intra-coronary          | EF, Perfusion, Infarct size   |                |
| Fernandez-Aviles et al | 2004 | Serial     | 20     | AMI | BM-MNCs | Intra-coronary     | EF                      |                               |                |
| Chen et al        | 56  | 2004        | RCT (Open label) | 69 | AMI      | BM-MNCs           | Intra-coronary          | EF                            |                |
| BOOST             | 60, 61 | 2004, 2006  | RCT (Open label) | 60 | AMI      | BM-MNCs           | Intra-coronary          | EF (transient)               |                |
| MAGIC Cell        | 62  | 2004        | Serial  | 27  | AMI      | BM-MNCs or EPCs   | Intra-coronary          | Feasible                     | Infarct size    |
| Janssens et al    | 59  | 2006        | RCT (Double-blind) | 67 | AMI      | BM-MNCs           | Intra-coronary          | EF                            |                |
| MAGIC Cell-3-DES  | 63  | 2006        | RCT     | 96  | AMI      | BM-MNCs           | Intra-coronary          | EF                            |                |
| REVIVAL-2         | 64  | 2006        | RCT (Double-blind) | 114| AMI      | G-CSF             | no cells                | Negative                     |                |
| REPAIR-AMI        | 57  | 2007        | RCT (Double-blind) | 204| AMI      | BM-MNCs           | Intra-coronary          | EF                            |                |
| ASTAMI            | 58  | 2007        | RCT (Double-blind) | 100| AMI      | BM-MNCs           | Intra-coronary          | EF                            |                |
| Stamm et al       | 69  | 2003        | Serial  | 12  | OMI      | EPCs              | Epicardial + CABG       | EF, Perfusion                |                |
| Herreros et al    | 70  | 2003        | Serial  | 12  | OMI      | Skeletal MB       | Epicardial + CABG       | EF, Regional contractility   |                |
| Siminiak et al    | 71  | 2004        | Serial  | 10  | OMI      | Skeletal MB       | Epicardial + CABG       | EF, Symptom                  | Sustained VT in 4 cases    |
| PONZAN            | 65  | 2005        | Serial  | 10  | OMI      | Skeletal MB       | Coronary sinus          | EF                            |                |
| IACT              | 66  | 2005        | Serial  | 18  | OMI      | BM-MNCs           | Intra-coronary          | EF, Symptom                  | In stent restenosis         |
| Katritsis et al   | 68  | 2005        | Serial  | 11  | OMI and AMI | EPCs + MSCs     | Intra-coronary          | Regional contractility       |                |
| Hendrix et al     | 69  | 2006        | RCT     | 20  | OMI      | BM-MNCs           | Epicardial + CABG       | Regional contractility       |                |
| TOPCARE-CHD       | 70  | 2006        | RCT (crossover) | 75 | AMI      | BM-MNCs or EPCs   | Intra-coronary          | EF, Regional contractility   |                |
| Tse et al         | 72  | 2003        | Serial  | 8   | Chronic IHD | BM-MNCs         | Trans-endocardial       | Regional function, Symptom   |                |
| Fuchs et al       | 73  | 2003        | Serial  | 10  | Chronic IHD | BM-MNCs         | Trans-endocardial       | Perfusion, Symptom           |                |
| Erbs et al        | 74  | 2005        | RCT (Double-blind) | 26 | Chronic IHD | EPCs             | Intra-coronary          | EF, Coronary flow reserve    |                |
| Perin et al       | 76  | 2003        | Serial  | 14  | ICM      | BM-MNCs           | Trans-endocardial       | EF, Perfusion, Symptom       | Sustained VT in 4 cases    |
| Hagège et al      | 76  | 2006        | Serial  | 10  | ICM      | Skeletal MB       | Epicardial + CABG       | EF, Regional contractility, Symptom |                |
| Dib et al         | 77  | 2005        | Serial  | 30  | ICM      | Skeletal MB       | Epicardial + CABG       | EF                            |                |

AMI: acute myocardial infarction, BM-MNC: bone marrow mononuclear cells, CABG: coronary artery bypass graft surgery, EF: ejection fraction, EPCs: endothelial progenitor cells, G-CSF: granulocyte colony stimulating factor, ICM: ischemic cardiomyopathy, IHD: ischemic heart disease, MB: myoblast, MSCs: mesenchymal stem cells, OMI: old myocardial infarction, PBSCT: peripheral blood stem cell transplantation, RCT: randomized clinical trial.
for the general status of such patients tends to be so poor to undergo general anesthesia during bone marrow aspiration. Thus, strategies that can result in similar effectiveness and safety with less invasiveness would be more ideal. One of the candidates for such method is the use of PB-MNCs. Because PB-MNCs contain few progenitor cells, an attempt to effectively mobilize them had been explored. There were several reports using PB-MNCs with increased contents of progenitors by the pre-administration of G-CSF. G-CSF increased the number of circulating CD34-positive progenitors by up to ten folds without major complications. These cells among with PB-MNCs were harvested by apheresis, condensed, and injected intramuscularly into critically ischemic limbs. The method had positive effects seemingly comparable to that obtained from BM-MNCs implantation. Recent basic studies revealed that the effect of cell therapy depends more on its ability to secrete growth factors (paracrine effect) than its incorporation into blood vessels. This nominates naïve PB-MNCs, which possess tremendous ability to induce paracrine effect, as an alternative to BM-MNCs or G-CSF administration. Clinical application of PB-MNCs for CLI was proven to induce relief from ischemia that is sufficient for limb salvage.

Several problems must be kept in mind when applying cells therapy for CLI. Because cell transplantation into large skeletal muscle mass results in systemic elevation of angiogenic factors, pathological angiogenesis such as worsening of diabetic retinopathy, neoplasm, or other unexpected complications may occur. Concerns do not limit to those practical concerns. How do these different cell types compare is largely unknown. Moreover, it is increasingly recognized that response to cell therapy on CLI differs according to the basal pathology of each patients. So far there has been only two randomized clinical trial on CLI. Thus, further investigations are needed to establish the safety and efficacy of, and to clarify the best components and target for, the cell therapy.

Acute Myocardial Infarction

Acute myocardial infarction (AMI) is a disease process involving robust necrosis of cardiomyocytes and endothelial cells, subsequent flaring and resolving of inflammation, and the resultant remodeling of the whole myocardium. Cell therapy thus have several points of application, including protection of otherwise dying cardiac cells, supplement of lost components of myocardium, and enhancement of healing and regenerating processes. In these regards various types of cells have been tested to treat experimental myocardial infarction, including BM-MNC, EPCs and MSCs. Now there are more than ten published clinical trials that have treated AMI by autologous cell therapy.

The first trial was reported in 2002, in which Strauer et al. recruited 20 serial patients with AMI. Five to 9 days after emergent balloon angioplasty and stent implantation, autologous bone marrow was aspirated from the ilium, and BM-MNCs were isolated. The cells were then infused directly into infarct-related coronary artery by a balloon catheter. Three months later they could find in the cell treated group significant decrease in infarct size in company with recovery of myocardial perfusion. They speculated that the marked therapeutic effect might be attributed to bone marrow cell-associated myocardial regeneration and neovascularization. Slightly later from this report, a trial comparing BM-MNCs and ex vivo expanded EPCs was published (TOPCARE-AMI). They treated 20 patients with reperfused AMI and performed intracoronary infusion of either bone marrow-derived or circulating blood-derived progenitor cells into the infarct related artery 4.3 ± 1.5 days after the onset. They reported that cell therapy, either by BM-MNCs or EPCs, improved ejection fraction, perfusion, and infarct size compared to a matched reference group. There were no adverse events related to cell transplantation during one-year follow up, providing excellent safety and feasibility profiles. Another candidate for myocardial regeneration is transplantation of MSCs. Chen et al. recruited 69 AMI patients with successful primary coronary intervention. BM-MNCs were harvested from these patients and were cultured for 10 days to obtain MSCs. MSCs or placebo saline were then infused into the infarct-related artery. Cell infusion resulted in improvement of left ventricular function. However requirement of GMP-grade cell processing center for the culture of MSCs limits its utility compared to the naïve BM-MNCs which requires less procedure with seemingly similar functional benefit.

Following these open label trials, several double blind, randomized clinical trials had taken place. The REPAIR-AMI group enrolled 204 AMI patients who were randomly assigned to receive BM-MNCs or placebo after successful reperfusion. Cell therapy benefited these cohorts significantly in improvement of LVEF at 4 months, especially for a group of patients with lower baseline function. This was associated with a reduction in the combined clinical end point of death, recurrence of myocardial infarction, and any revascularization procedure at one year, suggesting that cell therapy might be beneficial.
for the improvement of patient prognosis. However, other trials utilizing similar but slightly different protocol failed to show any benefit by BM-MNCs. The ASTAMI50 group differed to the REPAIR-AMI group in that they stored the cells overnight in un-buffered saline, which might have affected viability and bioactivities of the cells. Janssens et al.50 injected the cells within 24 hours of reperfusion therapy. Neither of these trials could show benefit of cell transplantation on myocardial function. Whether these differences had been critical, or whether beneficial effect of BM-MNCs transplantation would be reproducible, is presently unclear. A randomized controlled study (BOOST)60, 61 revealed that benefit of cell therapy was prominent in 6 months but not after 18 months, suggesting that cell therapy only accelerates recovery of LVEF. All these mixed results suggest the importance of designing the future study protocols by taking into account the precise mechanism of the disease and the therapy.

As done with the trials of limb ischemia, G-CSF mobilized peripheral blood stem cells were also applied to AMI. Kang et al.62 recruited 27 patients with revascularized AMI, and assigned them to either G-CSF mobilized cell infusion group, G-CSF alone group, and control group. Among them, cell infusion group showed improved cardiac function at 6 month. However the trial was discontinued due to high incidence of in-stent restenosis that occurred unexpectedly in the G-CSF treated patients. On the other hand, in the setting of drug eluting stent (DES) implantation, G-CSF showed no disadvantage in in-stent restenosis, while peripheral blood stem cell transplantation for AMI induced significant additive improvement in LVEF and remodeling.60 Interestingly, this cell therapy had no advantages in the setting of old myocardial infarction. Other group (REVIVAL-2)64 tested the value of systemic stem cell mobilization by G-CSF in patients with AMI in a randomized, double-blind, placebo-controlled trial recruiting 114 patients. They showed that G-CSF treatment without local stem cell infusion had no influence on infarct size or left ventricular function 4 to 6 months after treatment. The effect of GCSF administration nevertheless should not be underscored at this point. Because G-CSF has capacities to not only mobilize intrinsic stem cells, but also act directly onto cardiomyocytes to exhibit tremendous protective effect in the acute phase,65 the timing they applied G-CSF might have been too late to exhibit ideal results. Thus, more trial on this field should be awaited.

Old Myocardial Infarction

Although myocardial salvage by early reperfusion therapy has significantly improved mortality in patients with AMI, post-infarction heart failure resulting from ventricular remodeling remains a great problem. Although there is a great possibility that cell therapy in the acute phase may be beneficial on this issue, such procedures are carried out in quite a limited number of institutions. Thus, it would be ideal if such strategy had benefit on treating the sub-acute to chronic phase of myocardial infarction. There are some reports that dealt with this.

The IACT study66 recruited 18 patients with chronic myocardial infarction that had been treated with emergent direct coronary angioplasty, and treated them with intra-coronary infusion of BM-MNCs 5 months to 8.5 years after the onset of AMI. They found that BM-MNCs infusion successfully improved cardiac metabolism and function at three months. More recently, TOPCARE-CHD67 group accomplished crossover trial comparing BM-MNCs to EPCs in the treatment of stable ischemic heart disease who had had a myocardial infarction at least 3 months previously. They assessed cardiac function 3 months after each cell injections, and reported that transplantation of BM-MNCs was associated with moderate but significant improvement in the left ventricular ejection fraction.

The use of EPCs for old myocardial infarction seems less effective compared to BM-MNCs. TOPCARE-CHD67 crossover trial revealed that infusion of EPCs did not benefit cardiac function as did with the infusion of BM-MNC. Katritsis et al.68 enrolled patients with both recent and old anteroseptal myocardial infarction, and treated them with intra-coronary infusion of EPCs and MSCs. They showed that myocardial contractility of previously non-functioning myocardial segments improved, only in the AMI patients treated with the cells. Stamm et al.69 performed epicardial injection of EPCs in conjunction with coronary artery bypass grafting surgery, to treat patients with old myocardial infarction. Although they observed significant improvement in blood perfusion at the infarct sites, their contribution to actual improvement in left ventricular wall motion was modest.

Myoblasts can also be a good cell source to treat such patients. Herreros et al.70 recruited 12 patients with old myocardial infarction. They obtained autologous skeletal myoblasts by muscle biopsy, expanded them in culture with autologous serum for 3 weeks, and injected them from epicardium in conjunction with coronary artery bypass surgery. They concluded that this treatment is safe.
and feasible. Siminiak et al.\textsuperscript{71} reported similar study with evidence of functional recovery in ten patients with old myocardial infarction. Importantly, they experienced 4 cases of sustained ventricular tachycardia, which was prevented by prophylactic amiodarone infusion in the remaining 8 patients, giving a caution to the application of cell therapy for such patients.

**Chronic Myocardial Ischemia**

Owing to the strong potential to induce neovascularization, cell therapy can be applied for patients with chronic severe myocardial ischemia with no surgical option. Tse et al implanted autologous BM-MNC via catheter based trans-endocardial approach, to eight patients with stable angina refractory to maximum medical treatment.\textsuperscript{72} They found that cell implantation induced improvement in angina class, myocardial perfusion, and wall motion, without major adverse events. Fuchs et al.\textsuperscript{73} also used the same approach and had a similar result. Erbs et al.\textsuperscript{74} infused EPCs into 13 out of 26 recanalized chronic coronary total occlusion and found that this intervention improves coronary flow reserve in company with functional recovery of previously hibernating myocardium. Larger randomized clinical trials to investigate the effect of cell therapy in these settings are surely warranted.

**Heart Failure**

Myocardial infarction results in ventricular remodeling, which involves scar formation and chronically ischemic myocardium under insufficient perfusion, together with alteration of local and systemic humoral factors. As a consequence heart failure develops, which severely influences patient’s morbidity and mortality.

Implantation of skeletal myoblasts for patients with ischemic cardiomyopathy has extensively been studied. Following the first clinical experience of myoblast transplantation that seemed successful,\textsuperscript{75} a phase I clinical trial was taken place. Ten patients with postinfarction myocardial scar and severe left ventricular dysfunction (ejection fraction ≤ 35%) were enrolled to have injection of autologous myoblast in conjunction with coronary bypass surgery. Although this indicated significant improvement in cardiac function (mean New York Heart Association functional class improved from 2.7 ± 0.2 preoperatively to 1.6 ± 0.1 postoperatively, and ejection fraction increased from 24 ± 1% to 32 ± 1%), their patients showed delayed episodes of sustained ventricular tachycardia and were implanted with internal defibrillators. The long term follow up\textsuperscript{76} of these patients revealed durable efficacy on patients symptoms and myocardial function. Among 5 patients who received internal defibrillator for ventricular tachycardia, 3 patients received appropriate shocks for 3 arrhythmic storms at 6, 7, and 18 months after ACD implantation. Notably, no sustained ventricular tachycardia was observed among those patients with continuously improving ejection fraction, whereas 3 of 4 patients who exhibited sustained ventricular tachycardia showed a marked decrease in ejection fraction from short-term to long-term. The investigators speculate the possibility that those arrhythmic events were not related to the cell transplantation, but reflected the evolution of heart failure. Successful application of skeletal myoblasts were similarly shown by other groups\textsuperscript{77} who treated thirty patients undergoing concurrent coronary artery bypass grafting (CABG) or left ventricular assist device (LVAD) implantation. Left ventricular ejection fraction improved from 28% to 35% at 1 year and remained to 36% at 2 years. Histological evaluation in 4 of 6 patients who underwent heart transplantation in LVAD arm documented survival and engraftment of the skeletal myoblasts within the infarcted myocardium. These studies demonstrated that this modality offers a potential therapeutic treatment for end-stage heart disease.

Meanwhile, Perin et al.\textsuperscript{78} enrolled 21 patients with end-stage ischemic heart disease, whose left ventricular ejection fraction were less than 40% (mean 20%). Among them, 14 received trans-endocardial injection of BM-MNC into ischemic, but viable myocardium. Cell implantation resulted in blood flow recovery associated with global left ventricular function at 2 to 4 months after the treatment as seen in the ejection fraction improvement from baseline 20% to 29% at four months. Follow up report revealed that BM-MNCs injection significantly improved exercise capacity of the patients after 12 months.

Although the precise mechanism how these cells improved the function of severely ischemic myocardium is unknown, it is highly speculated that neovascularization might have played a major role. Because the development of heart failure in other types of cardiomyopathies is also thought to be a consequence of myocardial ischemia,\textsuperscript{79} it would be expected that cell therapy might have efficacy for end-stage heart failures other than ischemic cardiomyopathy.

**In Stent Restenosis**

Angioplasty is currently the major way to treat pro-
gressive atherosclerosis. The greatest shortcoming of angioplasty has been the restenosis, which occurs in consequence to endothelial denudation. EPCs capturing stents to accelerate the re-endothelialization was clinically applied to overcome this issue. HEALING-FIM trial registered 16 patients with de-novo coronary artery disease to be treated with CD-34 antibodies coated stents, and showed its safety and feasibility. This was followed by HEALING II trial that revealed its efficacy. Interestingly, there was a direct correlation between angiographic outcomes with EPCs titer, to which the use of statins had been a confounding variable. Because the functional properties of EPCs and other stem cells are known to be attenuated by various coronary risk factors, it is noteworthy to take into account the co-morbidity patients endure. Efforts to normalize, or even elevate the capacity of intrinsic stem cells will be an important issue regarding regenerative medicine in the near future.

PERSPECTIVES

One great controversy among cardiovascular intelligence is whether or not cell therapies should be applied into clinical application in the lack of precise knowledge regarding mechanistic link between donor cells and disease pathology. This argument became prominent ever since the stemness of these cells had been questioned one after another. Not to mention, any clinical trials without adequate translational research should be criticized ethically and scientifically. However, it is also needless to say that the true clinical mechanism can never be found without achieving relevant clinical investigations. There has never been a time that required so much attention to both bench-to-bedside and bedside-to-bench investigations. Close collaboration between clinicians and basic scientists definitely is the key to the future of cardiovascular therapeutics.

REFERENCES

1) Carmeliet P. Mechanisms of angiogenesis and arteriogenesis. Nat Med. 2000; 6: 389–95.
2) Müller P, Pfeiffer P, Koglin J, Schäfers HJ, Seeland U, Janzen I, et al. Cardiomyocytes of noncardiac origin in myocardial biopsies of human transplanted hearts. Circulation. 2002; 106: 31–5.
3) Orlic D, Kajstura J, Chimenti S, Jakoniuk I, Anderson SM, Li B, et al. Bone marrow cells regenerate infarcted myocardium. Nature. 2001; 410: 701–5.
4) Murry CE, Soonpa MH, Reinecke H, Nakajima H, Nakajima HO, Rubart M, et al. Haematopoietic stem cells do not transdifferentiate into cardiac myocytes in myocardial infarcts. Nature. 2004; 428: 664–8.
5) Balsam LB, Wagers AJ, Christensen JL, Kofidis T, Weissman IL, Robbins RC. Haematopoietic stem cells adopt mature haematopoietic fates in ischaemic myocardium. Nature. 2004; 428: 668–73.
6) Chien KR. Stem cells: lost in translation. Nature. 2004; 428: 607–8.
7) Bikfalvi A, Han ZC. Angiogenic factors are hematopoietic growth factors and vice versa. Leukemia. 1994; 8: 523–9.
8) Arras M, Ito WD, Scholz D, Winkler B, Schaper J, Schaper W. Monocyte activation in angiogenesis and collateral growth in the rabbit hindlimb. J Clin Invest. 1998; 101: 40–50.
9) Heil M, Ziegelhoeffer T, Mees B, Schaper W. A different outlook on the role of bone marrow stem cells in vascular growth: bone marrow delivers software not hardware. Circ Res. 2004; 94: 573–4.
10) Tateno K, Minamino T, Toko H, Akazawa H, Shimizu N, Takeda S, et al. Critical roles of muscle-secreted angiogenic factors in therapeutic neovascularization. Circ Res. 2006; 98: 1194–202.
11) Cho HJ, Lee N, Lee JY, Choi YJ, Li M, Wecker A, et al. Role of host tissues for sustained humoral effects after endothelial progenitor cell transplantation into the ischemic heart. J Exp Med. 2007; 204: 3257–69.
12) Laugwitz KL, Moretti A, Lam J, Gruber P, Chen Y, Woodard S, et al. Postnatal isl1+ cardioblasts enter fully differentiated cardiomyocyte lineages. Nature. 2005; 433: 647–53.
13) Matsuura K, Nagai T, Nishigaki N, Oyama T, Nishi J, Wada H, et al. Adult cardiac Sca-1-positive cells differentiate into beating cardiomyocytes. J Biol Chem. 2004; 279: 11384–91.
14) Torella D, Ellison GM, Karakikes I, Nadal-Ginard B. Growth-factor-mediated cardiac stem cell activation in myocardial regeneration. Nat Clin Pract Cardiovasc Med. 2007; 4 (Suppl 1): S46–51.
15) Mazhari R, Hare JM. Mechanisms of action of mesenchymal stem cells in cardiac repair: potential influences on the cardiac stem cell niche. Nat Clin Pract Cardiovasc Med. 2007; 4 (Suppl 1): S21–6.
16) Zhu W, Shiojima I, Ito Y, Li Z, Ikeda H, Yoshida M, et al. IGFBP-4 is an inhibitor of canonical Wnt signalling required for cardiogenesis. Nature. 2008; 454: 345–9.
17) Thomson JA, Itskovitz-Eldor J, Shapiro SS, Waknitz MA, Swiergiel JJ, Marshall VS, et al. Embryonic stem cell lines derived from human blastocysts. Science. 1998; 282: 1145–7.
18) Takahashi K, Tanabe K, Ohnuki M, Narita M, Ichisaka T, Tomoda K, et al. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. Cell. 2007; 131: 861–72.
19) Pittenger MF, Mackay AM, Beck SC, Jaiswal RK, Douglas R, Mosca JD, et al. Multilineage potential of
adult human mesenchymal stem cells. Science. 1999; 284: 143–7.

20) Makino S, Fukuda K, Miyoshi S, Konishi F, Kodama H, Pan J, et al. Cardiomyocytes can be generated from marrow stromal cells in vitro. J Clin Invest. 1999; 103: 697–705.

21) Shake JG, Gruber PJ, Baumgartner WA, Senechal G, Meyers J, Redmond JM, et al. Mesenchymal stem cell implantation in a swine myocardial infarct model: engraftment and functional effects. Ann Thorac Surg. 2002; 73: 1919–26.

22) Nygren JM, Jovinge S, Breitbach M, Säwén P, Röll W, Hescherle J, et al. Bone marrow-derived hematopoietic cells generate cardiomyocytes at a low frequency through cell fusion, but not transdifferentiation. Nat Med. 2004; 10: 494–501.

23) Gnecchi M, He H, Liang OD, Morello F, Mu H, et al. Paracrine action accounts for marked protection of ischemic heart by Akt-modified mesenchymal stem cells. Nat Med. 2005; 11: 367–8.

24) Asahara T, Murohara T, Sullivan A, Silver M, van der Zee R, Li T, et al. Isolation of putative progenitor endothelial cells for angiogenesis. Science. 1997; 275: 964–7.

25) Peichev M, Naiyer AJ, Pereira D, Zhu Z, Lane WJ, Williams M, et al. Expression of VEGFR-2 and AC133 by circulating human CD34 (+) cells identifies a population of functional endothelial precursors. Blood. 2000; 95: 952–8.

26) Asahara T, Masuda H, Takahashi T, Kalka C, Pastore C, Silver M, et al. Bone marrow origin of endothelial progenitor cells responsible for postnatal vasculogenesis in physiological and pathological neovascularization. Circ Res. 1999; 85: 221–8.

27) Kalka C, Masuda H, Takahashi T, Kalka-Moll WM, Silver M, Kearney M, et al. Transplantation of ex vivo expanded endothelial progenitor cells for therapeutic neovascularization. Proc Natl Acad Sci U S A. 2000; 97: 3422–7.

28) Kawamoto A, Gwon HC, Iwaguro H, Yamaguchi JI, Uchida S, Masuda H, et al. Therapeutic potential of ex vivo expanded endothelial progenitor cells for myocardial ischemia. Circulation. 2001; 103: 634–7.

29) Noishiki Y, Tomizawa Y, Yamane Y, Matsumoto A. Autocrine angiogenic vascular prosthesis with bone marrow transplantation. Nat Med. 1996; 2: 90–3.

30) Walter DH, Rittig K, Bahlmann FH, Kirchmair R, Silver M, Murayama T, et al. Statin therapy accelerates reendothelialization: a novel effect involving mobilization and incorporation of bone marrow-derived endothelial progenitor cells. Circulation. 2002; 105: 3017–24.

31) Bhattacharya V, McSweeney PA, Shi Q, Bruno B, Issida A, Nash R, et al. Enhanced endothelialization and microvesSEL formation in polyester grafts seeded with CD34 (+) bone marrow cells. Blood. 2000; 95: 581–5.

32) Vasa M, Fichtlscherer S, Aicher A, Adler K, Urbich C, Martin H, et al. Number and migratory activity of circulating endothelial progenitor cells inversely correlate with risk factors for coronary artery disease. Circ Res. 2001; 89: E1–7.

33) Tepper OM, Galiano RD, Capla JM, Kalka C, Gagne PJ, Jacobowitz GR, et al. Human endothelial progenitor cells from type II diabetics exhibit impaired proliferation, adhesion, and incorporation into vascular structures. Circulation. 2002; 106: 2781–6.

34) Jackson KA, Majka SM, Wang H, Pocius J, Hartley CJ, Majesky MW, et al. Regeneration of ischemic cardiac muscle and vascular endothelium by adult stem cells. J Clin Invest. 2001; 107: 395–402.

35) Ziegelhoeffer T, Fernandez B, Kostin S, Heil M, Voswinckel R, Helisch A, et al. Bone marrow-derived cells do not incorporate into the adult growing vasculature. Circ Res. 2004; 94: 230–8.

36) Ikenaga S, Hamano K, Nishida M, Kobayashi T, Li TS, Kobayashi S, et al. Autologous bone marrow implantation induced angiogenesis and improved deteriorated exercise capacity in a rat ischemic hindlimb model. J Surg Res. 2001; 96: 277–83.

37) Shintani S, Murohara T, Ikeda H, Ueno T, Sasaki K, Duan J, et al. Augmentation of postnatal neovascularization with autologous bone marrow transplantation. Circulation. 2001; 103: 897–903.

38) Kobayashi T, Hamano K, Li TS, Katoh T, Kobayashi S, Matsuzaki M, et al. Enhancement of angiogenesis by the implantation of self bone marrow cells in a rat ischemic heart model. J Surg Res. 2000; 89: 189–95.

39) Kamihata H, Matsuura H, Nishiue T, Fujiyama S, Tsutsuomi Y, Ozono R, et al. Implantation of bone marrow mononuclear cells into ischemic myocardium enhances collateral perfusion and regional function via side supply of angioblasts, angiogenic ligands, and cytokines. Circulation. 2001; 104: 1046–52.

40) Fuchs S, Baffour R, Zhou YF, Shou M, Pierre A, Tio FO, et al. Transendocardial delivery of autologous bone marrow enhances collateral perfusion and regional function in pigs with chronic experimental myocardial ischemia. J Am Coll Cardiol. 2001; 37: 1726–32.

41) Kocher AA, Schuster MD, Szabolcs MJ, Takuma S, Burkhoff D, Wang J, et al. Neovascularization of ischemic myocardium by human bone-marrow-derived angioblasts prevents cardiomyocyte apoptosis, reduces remodeling and improves cardiac function. Nat Med. 2001; 7: 430–6.

42) Rehman J, Li J, Orcscheil CM, March KL. Peripheral blood “endothelial progenitor cells” are derived from monocyte/macrophages and secrete angiogenic growth factors. Circulation. 2003; 107: 1164–9.

43) Iba O, Matsubara H, Nozawa Y, Fujiyama S, Amano K, Mori Y, et al. Angiogenesis by implantation of peripheral blood mononuclear cells and platelets into ischemic limbs. Circulation. 2002; 106: 2019–25.

44) Kamihata H, Matsuura H, Nishiue T, Fujiyama S, Amano K, Iba O, et al. Improvement of collateral perfusion and regional function by implantation of pe-
Peripheral blood mononuclear cells into ischemic hibernating myocardium. Arterioscler Thromb Vasc Biol. 2002; 22: 1804–10.

45) Jain M, DerSimonian H, Brenner DA, Ngoy S, Teller P, Edge AS, et al. Cell therapy attenuates deleterious ventricular remodeling and improves cardiac performance after myocardial infarction. Circulation. 2001; 103: 1920–7.

46) Menasché P. Skeletal muscle satellite cell transplantation. Cardiovasc Res. 2003; 58: 351–7.

47) Tateishi-Yuyama E, Matsubara H, Murohara T, Ikeda U, Shintani S, Masaki H, et al. Therapeutic angiogenesis for patients with limb ischaemia by autologous transplantation of bone-marrow cells: a pilot study and a randomised controlled trial. Lancet. 2002; 360: 427–35.

48) Ishida A, Ohyu Y, Sakuda H, Ohshiro K, Higashuesato Y, Nakaya M, et al. Autologous peripheral blood mononuclear cell implantation for patients with peripheral arterial disease improves limb ischemia. Circ J. 2005; 69: 1260–5.

49) Huang P, Li S, Han M, Xiao Z, Yang R, Han ZC. Autologous transplantation of granulocyte colony-stimulating factor-mobilized peripheral blood mononuclear cells improves critical limb ischemia in diabetes. Diabetes Care. 2005; 28: 2155–60.

50) Minamino T, Toko H, Tateno K, Nagaya N, Akutsu K, Chikuni M, Kamei M, et al. Unblinded pilot study of autologous transplantation of bone marrow mononuclear cells in patients with thrombosis. Circulation. 2006; 114: 2679–84.

51) Miyamoto K, Nishigami K, Nagaya N, Akutsu K, Chikuni M, Kamei M, et al. Safety and efficacy of autologous progenitor cell transplantation for therapeutic angiogenesis in patients with critical limb ischemia. Circ J. 2007; 71: 196–201.

52) Strauer BE, Brehm M, Zeuthen T, Köstering M, Hernandez A, Sorg RV, et al. Repair of infarcted myocardium by autologous intracoronary mononuclear bone marrow cell transplantation in humans. Circulation. 2002; 106: 1913–8.

53) Assmus B, Schächinger V, Teupe C, Britten M, Lehmann R, Döbert N, et al. Transplantation of progenitor cells and regeneration enhancement in acute myocardial infarction (TOPCARE-AMI). Circulation. 2002; 106: 3009–17.

54) Schächinger V, Assmus B, Britten MB, Honold J, Lehmann R, Teupe C, et al. Transplantation of progenitor cells and regeneration enhancement in acute myocardial infarction: final one-year results of the TOPCARE-AMI Trial. J Am Coll Cardiol. 2004; 44: 1690–9.

55) Chen SL, Fang WW, Ye F, Liu YH, Qian J, Shan SJ, et al. Effect on left ventricular function of intracoronary transplantation of autologous bone marrow mesenchymal stem cell in patients with acute myocardial infarction. Am J Cardiol. 2004; 94: 92–5.

56) Schächinger V, Erbs S, Elsässer A, Haberbosch W, Hambrecht R, Hölscermann H, et al. Intracoronal bone marrow-derived progenitor cells in acute myocardial infarction. N Engl J Med. 2002; 355: 1210–21.

57) Lunde K, Solheim S, Aakhus S, Arnesen H, Abdelnoor M, Egeland T, et al. Intracoronal injection of mononuclear bone marrow cells in acute myocardial infarction. N Engl J Med. 2006; 355: 1199–209.

58) Janssens S, Dubois C, Boggaert J, Theunissen K, Deroose C, Desmet W, et al. Autologous bone marrow-derived stem-cell transfer in patients with ST-segment elevation myocardial infarction: double-blind, randomised controlled trial. Lancet. 2006; 367: 113–21.

59) Strauer BE, Brehm M, Zeus T, Bartsch T, Schannwell H, et al. Therapeutic angiogenesis for patients with peripheral artery disease: the IACT Study. J Am Coll Cardiol. 2005; 46: 113–21.

60) Meyer GP, Wollert KC, Lotz J, Ringes- Lichtenberg S, Lippolt C, Breidenbach C, et al. Intracoronal autologous bone-marrow cell transfer after myocardial infarction: the BOOST randomised controlled clinical trial. Lancet. 2004; 364: 141–8.

61) Meyer GP, Wollert KC, Lotz J, Steffens J, Lippolt P, Fichtner S, et al. Intracoronary bone marrow cell transfer after myocardial infarction: eighteen months follow-up data from the randomized, controlled BOOST (Bone marrow transfer to enhance ST-elevation infarct regeneration trial). Circulation. 2006; 113: 1287–94.

62) Kang HJ, Kim HS, Zhang SY, Park KW, Cho HJ, Koo BK, et al. Effects of intracoronary infusion of peripheral blood stem-cell mobilised with granulocyte-colony stimulating factor on left ventricular systolic function and restenosis after coronary stenting in myocardial infarction: the MAGIC cell randomised clinical trial. Lancet. 2004; 363: 751–6.

63) Kang HJ, Lee HY, Na SH, Chang SA, Park KW, Kim HK, et al. Differential effect of intracoronary infusion of mobilized peripheral blood stem cells by granulocyte colony-stimulating factor on left ventricular function and remodeling in patients with acute myocardial infarction versus old myocardial infarction: the MAGIC Cell-3-DES randomized, controlled trial. Circulation. 2006; 114 (1 Suppl): I145–51.

64) ZohlnéDER D, Ott I, Mehilli J, Schömig K, Michalk F, Ibrahim T, et al. Stem cell mobilization by granulocyte colony-stimulating factor in patients with acute myocardial infarction: a randomized controlled trial. JAMA. 2006; 295: 1003–10.

65) Harada M, Qin Y, Takano H, Minamino T, Zou Y, Toko H, et al. G-CSF prevents cardiac remodeling after myocardial infarction by activating the Jak-Stat pathway in cardiomycocytes. Nat Med. 2005; 11: 305–11.

66) Strauer BE, Brehm M, Zeuthen T, Barschtsch T, Schannwell C, Antke C, et al. Regeneration of human infarcted heart muscle by intracoronary autologous bone marrow cell transplantation in chronic coronary artery disease: the IACT Study. J Am Coll Cardiol. 2005; 46: 1651–8.
67) Assmus B, Honold J, Schächinger V, Britten MB, Fischer-Rasokat U, Lehmann R, et al. Transcoronary transplantation of progenitor cells after myocardial infarction. N Engl J Med. 2006; 355: 1222–32.
68) Katritsis DG, Sotiropoulou PA, Karvouni E, Karabinos I, Korovesis S, Perez SA, et al. Transcoronary transplantation of autologous mesenchymal stem cells and endothelial progenitors into infarcted human myocardium. Catheter Cardiovasc Interv. 2005; 65: 321–9.
69) Stamm C, Westphal B, Kleine HD, Petzsch M, Kittner C, Klinge H, et al. Autologous bone-marrow stem-cell transplantation for myocardial regeneration. Lancet. 2003; 361: 45–6.
70) Herreros J, Prósper F, Perez A, Gavira JJ, Garcia-Velloso MJ, Barba J, et al. Autologous intramyocardial injection of cultured skeletal muscle-derived stem cells in patients with non-acute myocardial infarction. Eur Heart J. 2003; 24: 2012–20.
71) Siminiak T, Kalawski R, Fiszer D, Jerzykowska O, Rzeźniczak J, Rozwadowska N, et al. Autologous skeletal myoblast transplantation for the treatment of postinfarction myocardial injury: phase I clinical study with 12 months of follow-up. Am Heart J. 2004; 148: 531–7.
72) Tse HF, Kwong YL, Chan JK, Lo G, Ho CL, Lau CP. Angiogenesis in ischaemic myocardium by intramyocardial autologous bone marrow mononuclear cell implantation. Lancet. 2003; 361: 47–9.
73) Fuchs S, Satler LF, Kornowski R, Okubagzi P, Weisz G, Baffour R, et al. Catheter-based autologous bone marrow myoblast injection in no-option patients with advanced coronary artery disease: a feasibility study. J Am Coll Cardiol. 2003; 41: 1721–4.
74) Erbs S, Linke A, Adams V, Lenk K, Thiele H, Diederich KW, et al. Transplantation of blood-derived progenitor cells after recanalization of chronic coronary artery occlusion: first randomized and placebo-controlled study. Circ Res. 2005; 97: 756–62.
75) Menasché P, Hagège AA, Scorsin M, Pouzet B, Desnos M, Duboc D, et al. Myoblast transplantation for heart failure. Lancet. 2001; 357: 279–80.
76) Hagège AA, Marolleau JP, Vilquin JT, Alhérétique A, Peyrand S, Duboc D, et al. Skeletal myoblast transplantation in ischemic heart failure: long-term follow-up of the first phase I cohort of patients. Circulation. 2006; 114 (1 Suppl): I108–13.
77) Dib N, Michler RE, Pagani FD, Wright S, Kereiakes DJ, Lengerich R, et al. Safety and feasibility of autologous myoblast transplantation in patients with ischemic cardiomyopathy: four-year follow-up. Circulation. 2005; 112: 1748–55.
78) Perin EC, Dohmann HF, Borovevic R, Silva SA, Sousa AL, Mesquita CT, et al. Transendocardial, autologous bone marrow cell transplantation for severe, chronic ischemic heart failure. Circulation. 2003; 107: 2294–302.
79) Sano M, Minamino T, Toko H, Miyauchi H, Orimo M, Qin Y, et al. p53-induced inhibition of Hif-1 causes cardiac dysfunction during pressure overload. Nature. 2007; 446: 444–8.
80) Aoki J, Serruys PW, van Beusekom H, Ong AT, McFadden EP, Sianos G, et al. Endothelial progenitor cell capture by stents coated with antibody against CD34: the HEALING-FIM (Healthy Endothelial Accelerated Lining Inhibits Neointimal Growth-First In Man) Registry. J Am Coll Cardiol. 2005; 45: 1574–9.
81) Silber S. Capturing circulating endothelial progenitor cells: a new concept tested in the HEALING studies. Minerva Cardioangiol. 2006; 54: 1–3.