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# Stem Cell Therapy: A New Perspective in the Treatment of Patients with Acute Myocardial Infarction

G. P. Meyer, K. C. Wollert, H. Drexler

Department of Cardiology and Angiology, Hannover Medical School, Germany

**Abstract**
Experimental studies suggest that cardiac transfer of stem and progenitor cells can have a favorable impact on tissue perfusion and contractile performance after acute myocardial infarction (AMI). While the mechanistic background of stem cell therapy is still intensely debated, the concept of cell therapy has already been introduced into the clinical setting, where small, mostly uncontrolled trials indicate that stem cell therapy may be feasible in patients. The overall clinical experience also suggests that stem cell therapy can be safely performed, if the right cell type is used in the right clinical setting. Preliminary efficacy data indicate that stem cells have the potential to enhance myocardial perfusion and/or contractile performance in patients with AMI. The field now is rapidly moving towards intermediate-size, double-blinded trials to gather more safety and efficacy data. Ultimately, large outcome trials will have to be conducted. At the same time, continued basic research to elucidate the underlying mechanism of stem cell therapy is needed.

*Key words:* myocardial infarction, stem cells, intracoronary therapy

## Introduction

Cardiovascular diseases account for the majority of deaths in Europe with half of these caused by coronary artery disease (CAD). Once CAD has led to heart failure, the prognosis is poor, even compared to patients suffering from cancer. About 50% of patients with heart failure will die within 5 years after the diagnosis has been made. Modern reperfusion strategies like percutaneous coronary intervention (PCI) with stent implantation and advances in pharmacological management have improved short and long term outcomes, however, they also resulted in an increasing proportion of AMI survivors at heightened risk of developing adverse LV remodeling and heart failure. Today, none of the available therapies addresses the underlying cause of the remodeling process, i.e. the damage of cardiomyocytes and vasculature in the infarcted area. Therefore, strategies aiming at regeneration could represent a future solution to this problem. In this respect, it is of special interest that a fraction of cardiomyocytes may be able to reenter the cell-cycle and that limited regeneration can occur through recruitment of resident and circulating stem cells [1]. These endogenous repair mechanisms are overwhelmed in patients with AMI or advanced coronary artery disease. However, the existence of endogenous repair mechanisms suggests that cardiac repair may be achieved therapeutically in these clinical settings. Another strategy of AMI-repair is following the concept of adult stem cell plasticity [2, 3]. Stem cells are capable of self-renewal, transformation into dedicated progenitor cells, and differentiation into specialized progeny. Plasticity implies that stem cells can transdifferentiate into mature cell types outside their original lineage in response to microenvironmental cues. For example, hematopoietic stem cells (HSCs), when transplanted into the (murine) myocardium, may transdifferentiate into cardiomyocytes and blood vessels thereby improving heart function and survival [4]. However, the mechanistic underpinnings of stem cell therapy appear to be far more complex than previously anticipated. It has been proposed that stem cells release angiogenic ligands, protect cardiomyocytes from apoptotic cell death, induce proliferation of endogenous cardiomyocytes, and may recruit resident cardiac stem cells [5-9]. Regardless of the mechanisms, there appears to be general agreement that stem cell therapy has the potential to improve perfusion and contractile performance of the injured heart [4-7, 9, 10].

## Potential Donor Cells

A variety of stem and progenitor cell populations could be used for cardiac repair. Each cell type has its own profile of advantages, limitations, and practicability issues in specific clinical settings. Studies comparing the regenerative capacity of distinct cell populations are scarce. Many investigators have therefore chosen a pragmatic approach by using unfractionated bone marrow cells (BMCs) [11-22], which contain different stem and progenitor cell populations, including HSCs, endothelial progenitor cells (EPCs), and mesenchymal stem cells (MSCs). Ease of harvest and lack of extensive requirement for ex vivo manipulation are additional advantages of using unselected BMCs.

### *Endothelial Progenitor Cells*

EPCs have originally been defined by their cell surface expression of the hematopoietic marker proteins CD133 and CD34 and the endothelial marker vascular endothelial growth factor receptor-2, and their capacity to incorporate into sites of neovascularization and to differentiate into endothelial cells *in situ*.[23] Increasing evidence suggests that culture-expanded EPCs also

contain a CD14+/CD34--mononuclear cell population with "EPC-capacity", which mediates its angiogenic effects by releasing paracrine factors [24, 25]. Notably, EPC numbers and their angiogenic capacity are impaired in patients with coronary artery disease, which may limit their therapeutic usefulness [26, 27].

*CD133+ Cells*

The cell surface antigen CD133 is expressed on early HSCs and EPCs, both of which collaborate to promote vascularization of ischemic tissues [28]. CD133+ cells can integrate into sites of neovascularization and differentiate into mature endothelial cells. Since CD133 expression is lost on myelomonocytic cells, this marker provides an effective means to distinguish "true" CD133+ EPCs from EPCs of myelomonocytic origin [24]. Less than 1% of nucleated BMCs are CD133+, and as these cells cannot be expanded ex vivo, only limited numbers of CD133+ cells can be obtained for therapeutic purposes.

*Mesenchymal Stem Cells*

MSCs represent a rare population of CD34- and CD133- cells present in bone marrow stroma and other mesenchymal tissues [29]. MSCs can readily differentiate into osteocytes, chondrocytes, and adipocytes. Differentiation of MSCs to cardiomyocyte-like cells has been observed under specific culture conditions and after injection into healthy or infarcted myocardium in animals [30-32]. When injected into infarct tissue, MSCs may enhance regional wall motion and prevent remodeling of the remote, non-infarcted myocardium [32, 33]. Little is known about the effects of MSCs on myocardial perfusion. It is interesting to note however, that cultured MSCs secrete angiogenic cytokines, that improve collateral blood flow recovery in a murine hind limb ischemia model [8].

*Skeletal Myoblasts*

Skeletal myoblasts, are progenitor cells that can be isolated from skeletal muscle biopsies and expanded in vitro. Myoblasts differentiate into myotubes and retain skeletal muscle properties when transplanted into an infarct scar [34-37]. Although myotubes do not couple with resident cardiomyocytes electromechanically, myoblast transplantation has been shown to augment systolic and diastolic performance in animal models of myocardial infarction [38].

*Resident cardiac stem cells*

The presence of resident cardiac stem cell (CSC) population(s) capable of differentiating into cardiomyocyte or vascular lineages suggests that these cells could be used for cardiac tissue repair [39-43]. CSCs can be clonally expanded from human myocardial biopsies [43]. It has been reported that intramyocardial injection of these cells after AMI in mice promotes cardiomyocyte and vascular cell formation and leads to an improvement in systolic function [43]. If these findings can be reproduced, CSCs hold great promise for clinical applications, although, it is conceivable that the bone marrow may contain a stem cell population with similar properties [44].

*Embryonic stem cells*

Embryonic stem (ES) cells are totipotent stem cells derived from the inner cell mass of blastocysts. Under specific culture conditions, ES cells differentiate into multicellular embryoid bodies containing differentiated cells from all three germ layers including cardiomyocytes. Human ES cell-derived cardiomyocytes display structural and functional properties of early-stage cardiomyocytes [45, 46]. In theory, infinite numbers of cardiomyocytes could be obtained from human ES cell clones. However, unresolved ethical and legal issues, concerns about the tumorigenicity of the cells, and the need to use allogeneic cells for transplantation currently limit their use in clinical studies. Eventually, nuclear transfer techniques may provide a means for generating an unlimited supply of histocompatible ES cells for the treatment of cardiac disease [47].

## Modes of Cell Delivery

The goal of any cell delivery strategy is to transplant sufficient numbers of cells into the myocardial region of interest and to achieve maximum retention of cells within that area. Transvascular strategies are especially suited for the treatment of recently infarcted and reperfused myocardium when chemoattractants and cell adhesion molecules are highly expressed [48-50]. Because the application of stem cell therapy shortly after AMI is limited to non-surgical approaches, we will discuss the intracoronary- and intravenous infusion and mobilization strategies.

*Intracoronary Artery Infusion*

Selective intracoronary application delivers a maximum concentration of cells homogeneously to the site of injury during first passage. Unselected BMCs, circulating blood-derived progenitors cells, and MSCs have been delivered via the intracoronary route in patients with AMI (Table 1). In these studies, cells were delivered through the central lumen of an over-the-wire balloon catheter during transient balloon inflations to maximize the contact time of the cells with the microcirculation of the infarct-related artery. In the hands of an experienced operator, intracoronary delivery is relatively easy to perform within less than an hour.

*Intravenous Infusion*

In experimental models, intravenous delivery of EPCs or MSCs has been shown to improve cardiac function after AMI [5, 29, 51]. However, homing of cells to non-cardiac organs limits the clinical applicability of this approach [52, 53]. In a recent study in post AMI patients, significant myocardial homing of unselected BMCs was observed only after intracoronary stop-flow delivery but not after intravenous application [54].

*Mobilization of Stem and Progenitor Cells*

Considering that the acutely infarcted myocardium recruits circulating stem and progenitor cells to the site of injury [5, 51, 55, 56], stem and progenitor cell mobilization by cytokines may offer a non-invasive strategy for cardiac regeneration. This concept has been tested in animal models of AMI [57-61], and in pilot studies in patients with AMI and chronic myocardial ischemia [62, 63].

*Table 1.* Intracoronary Cell Therapy Trials in Patients with Acute Myocardial Infarction

| Study | [n] | Cell Type | Dose | Time after AMI | Outcomes | |
|---|---|---|---|---|---|---|
| | | | | | Improved | No Change |
| Strauer et al.<br>(Ref. [11]) | 10 treated<br>10 controls* | MNC | $2.8\pm2.2\times10^{7}$ | 5-9 days | Regional wall motion#<br>Infarct size ↓<br>Perfusion# | Global LVEF<br>LVEDV# |
| TOPCARE-AMI<br>(Refs. [12-14]) | 29 MNC<br>30 CPC<br>11 controls* | MNC<br>CPC | $2.1\pm0.8\times10^{8}$<br>$1.6\pm1.2\times10^{7}$ | 5±2 days | Regional wall motion#<br>Global LVEF#<br>Infarct size ↓#<br>Coronary flow# | LVEDV# |
| Fernandez-Aviles et al.<br>(Ref. [15]) | 20 treated<br>13 controls* | MNC | $7.8\pm4.1\times10^{7}$ | 14±6 days | Regional wall motion#<br>Global LVEF# | LVEDV# |
| Kuethe et al.<br>(Ref. [16]) | 5 treated | MNC | $3.9\pm2.3\times10^{7}$ | 6 days | --- | Regional wall motion#<br>Global LVEF# |
| BOOST<br>(Ref. [17]) | 30 treated<br>30 controls | NC | $2.5\pm0.9\times10^{9}$ | 6±1 days | Regional wall motion<br>Global LVEF | LVEDV<br>Infarct size |
| Chen et al.<br>(Ref. [76]) | 34 treated<br>35 controls | MSC | $4.8\pm6.0\times10^{10}$ | 18 days | Regional wall motion<br>Global LVEF<br>Infarct size ↓<br>LVEDV ↓ | --- |
| Vanderheyden et al.<br>(Ref. [78]) | 12 treated<br>10 controls* | $CD133^{+}$ | $6.6\pm1.4\times10^{6}$ | 14±6 days | Regional wall motion#<br>Global LVEF#<br>Perfusion# | --- |

MNC denotes bone marrow-derived mononuclear cells; CPC, circulating blood-derived progenitor cells; NC, bone marrow-derived nucleated cells; MSC, bone marrow-derived mesenchymal stem cells; $CD133^{+}$, bone marrow-derived $CD133^{+}$ cells; i.c., intracoronary; AMI, acute myocardial infarction; LVEF, left ventricular ejection fraction; LVEDV, left ventricular end-diastolic volume; *, non-randomized control groups; #, effects reported only within cell therapy groups. Values are means ± SD.

*Transendocardial Injection*
Using an injection needle catheter advanced across the aortic valve and positioned against the endocardial surface, cells can be directly injected into the left ventricular (LV) wall [19-22, 64]. Electromechanical mapping of the endocardial surface can be used to delineate viable, ischemic, and scarred myocardium before cell injections. Average mapping and injection procedure times between 60 and 200 minutes have been reported [19-22].

*Transcoronary Vein Injection*
A catheter system incorporating an ultrasound tip for guidance and an extendable needle for myocardial access has been used to deliver BMCs through the coronary veins into normal pig myocardium and in a pilot trial in patients with ischemic cardiomyopathy [65, 66].

## Experimental Background of Stem Cell Therapy after AMI

In one of the earliest studies, HSCs were injected into the infarct border zone after coronary artery ligation in mice. Several days later, the infarcted area was replaced by newly formed myocardium with HSC-derived myocytes and vascular structures [4]. Transdifferentiation to cardiomyocytes and vascular structures has also been reported after transfer of $CD34^+$ cells into mice with AMI [56]. Recent studies questioning that HSCs can transdifferentiate to cardiomyocytes when transplanted into infarcted murine myocardium have ignited a heated debate [10, 67, 68]. Yet, while data have been presented to support and to refute this idea, both sides agree that HSC transplantation can improve cardiac function after AMI [4, 10]. Improvement of cardiac function has also been observed after transplantation of unselected BMCs or EPCs. Although myocyte formation did not occur, cells were shown to secrete angiogenic ligands, to incorporate into foci of neovascularization, and to improve regional capillarization and blood flow [6, 51, 69].

## Clinical Trial Experience

Several trials were initiated to test whether cell therapy is safe and feasible in patients after AMI. Some have decried the clinical trials as being premature without a more complete understanding of the underlying mechanisms [68], while others have pointed out that the clinical trials are justified by the potential benefits of cell therapy [70]. All clinical studies included patients with AMI who had undergone primary angioplasty and stent implantation to reopen the infarct-related artery, and cells were infused intracoronarily by using the stop-flow balloon-catheter approach. In this regard, the clinical studies differ significantly from the animal studies, where the infarct-related artery was not reperfused and cells were directly injected into the myocardium [4, 6, 10, 51]. The clinical trials may be categorized into studies using unselected BMCs or selected cell populations (Table 1).

*Unselected Bone Marrow Cells*
The combined experience from more than 100 patients suggests that intracoronary delivery of unselected BMCs (all nucleated cells or mononuclear cell fraction only) is safe in the short- and mid-term (several months) [11, 12, 14-17]. No bleeding complications were noted after bone marrow harvest. Intracoronary BMC infusions did not appear to inflict additional ischemic damage to the myocardium or to promote a systemic inflammatory reaction, since no further increases in serum troponin or CRP levels were observed. No increased rates of in-stent restenosis were observed after transfer of unselected BMCs [14, 15, 17]. One patient developed in-stent thrombosis of the target vessel three days after cell infusion, although unlikely, it cannot be excluded that this complication was somehow related to cell therapy [14]. Clinical surveillance, Holter monitoring, and data from an electrophysiological study indicate that intracoronary BMC-transfer is not associated with an increased propensity to ventricular or supraventricular arrhythmias [11, 12, 14-17]. Direct injection of filtered nucleated BMCs into the acutely infarcted myocardium in rats has been found to induce intramyocardial calcifications [71]. No evidence for intramyocardial calcifications or tumor formation has been obtained in patients 12-18 months after intracoronary delivery of Ficoll or gelatine gradient-purified BMCs [14, 72].

Except for one study that included only five patients and no control group [16], all trials indicate that intracoronary transfer of unselected BMCs enhances regional wall motion in the infarcted area [11, 12, 15, 17]. In the three largest studies, this was associated with an increase also in global LVEF [12, 15, 17]. In contrast to earlier trials which included non-randomized control groups [11, 12, 15], the BOne marrOw transfer to enhance ST-elevation infarct regeneration (BOOST) trial included a randomized control group [17]. In the BOOST trial, BMC-transfer resulted in an improvement of LVEF of six percentage points as compared to the control group after six months. For comparison, improvements of three to four percentage points are achieved by primary angioplasty and stent implantation in AMI and this results in better clinical outcomes as compared to thrombolytic strategies [73, 74]. Improvement of LVEF was due mostly to improved regional wall motion in the infarct border zone [17]. Importantly, the effects of BMC-transfer were observed on top of the benefits associated with established interventional and medical strategies to promote functional recovery after AMI [17]. In contrast to earlier non-randomized studies [11, 12], a significant reduction of infarct size was not observed in the BOOST trial [17]. However, larger trials are required to further clarify the issue whether formation of new muscle tissue can be achieved by BMC-transfer. So far, no trial has demonstrated a significant effect of BMC-transfer on LV end-diastolic volumes, suggesting that unselected BMCs may have a limited impact on LV remodeling after AMI [11 12, 15-17]. Follow-up data from the BOOST trial show that the improvement of LVEF is maintained after 18 months and indicate that BMC-transfer prevents progression of diastolic dysfunction after AMI [72, 75].

*Selected Bone Marrow Cell Populations*
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(TOPCARE-AMI) trial compared unselected mononuclear BMCs with circulating blood-derived progenitor cells (mostly EPCs). Both cell types appeared to have similar safety and efficacy profiles [12, 14].

The therapeutic effects of MSC transplantation after AMI have been investigated in one clinical trial [76]. No arrhythmias or other side effects were observed. Unfortunately, it was not reported whether intracoronary MSC delivery promoted ischemic damage to the myocardium [76], a complication that has occurred after intracoronary MSC infusions in dogs [77]. Six months after MSC-transfer, regional wall motion and global LVEF were improved and LV end-diastolic volume was decreased compared to a randomized control group that had received an intracoronary infusion of saline [76].

In another clinical trial using selected BMC populations, $CD133^+$ cells were infused into the infarct-related artery [78, 79]. After four months, six out of 14 patients had developed a significant in-stent restenosis or complete re-occlusion, and two had developed a de novo lesion in the infarct-related artery [79]. These numbers are worrisome, but the study may be too small to establish that these side effects are causally related to $CD133^+$ cell transfer. Global LVEF, regional wall motion, and tissue perfusion increased in the cell transfer group but not in a cohort of matched control patients [78]. However, firm conclusions regarding efficacy cannot be derived from this small pilot trial.

*Stem and Progenitor Cell Mobilization*

Stem cell mobilization with stem cell factor (SCF) and/or granulocyte colony-stimulating factor (G-CSF) have been proposed to stimulate myogenesis and angiogenesis in the infarcted area and to improve cardiac function after AMI in mice [57, 58]. By contrast, treatment with SCF and G-CSF enhances vascularization of the infarcted area but does not improve cardiac function in baboons after AMI [61]. Perhaps, reperfusion of the infarct-related artery prior to cytokine therapy would have permitted better access of mobilized cells to the infarct center in this large animal model [61]. Of note, G-CSF may accelerate infarct healing by enhancing macrophage infiltration and matrix metalloproteinase activation [60], and suppress cardiomyocyte apoptosis by activating the cytoprotective STAT3 transcription factor [80], suggesting that stem cell-independent mechanisms may contribute to the effects of G-CSF after AMI.

In a first clinical investigation, 10 patients presenting with myocardial infarction 2 to 270 days after symptom onset were treated with G-CSF at 10 mg/kg body weight for four days. Patients then underwent angioplasty and stent implantation of the infarct-related artery. In seven of these patients, G-CSF mobilized peripheral blood-derived leukocytes were collected just prior to the intervention and infused into the infarct-related artery after stent placement. No deaths, substantial arrhythmias, aggravation of heart failure, or angina occurred during G-CSF administration and a six month follow-up period. However, cell infusions resulted in a 65% increase in serum creatine kinase-MB levels, indicative of mild myocardial damage. More seriously, seven of the 10 patients developed in-stent restenosis at six months, which prompted a premature termination of the study [81]. It should be pointed out that vascular injury by balloon angioplasty and stenting had been performed in this study while systemic leukocyte counts were greatly elevated. G-CSF has the potential to activate neutrophils, for example by stimulating adhesion to endothelial cells thereby influencing their recruitment at sites of inflammation and tissue injury [82]. These systemic effects of G-CSF may have contributed to excess neointima proliferation and restenosis. Although an improvement in LVEF was observed in patients receiving G-CSF and cell infusions [81], interpretation of this finding is impossible without a control group. In a more recent study, 15 patients with AMI were treated with G-CSF at 10 mg/kg body weight for six days, starting $80 \pm 30$ min after primary angioplasty and stent implantation of the infarct-related artery [62, 83]. G-CSF treatment after stent implantation was not associated with an enhanced rate of in-stent restenosis, or other serious adverse events [83]. Compared to a randomized control group, patients receiving G-CSF experienced a more pronounced recovery of global LVEF after four months. However, the beneficial effects of G-CSF were magnified by an unexpected decrease in LVEF in the control group [62].

## Summary and Future Research

In summary, preliminary clinical evidence suggests that stem cell therapy might work after AMI. While the initial clinical studies have generated a great deal of hope, we should take into account the lessons learned from the translation of therapeutic angiogenesis into clinical studies, where great expectations raised by open studies have not been confirmed by subsequent randomized trials. There is a lot of work to be done. Upcoming trials (randomized-controlled, double-blind) should address procedural issues such as the optimal cell type, cell dosage, timing of cell transfer, and the use of combined strategies, like the use of cytokines. Furthermore, specific subgroups need to be prospectively defined in future trials, e.g. patients presenting late after symptom onset in whom little myocardial salvage can be expected from reperfusion therapy [84]. Patients with AMI and heart failure symptoms have been excluded from previous trials. Considering that these patients may benefit most from an improvement in LVEF, future trials should assess the effects of cell therapy in this patient subgroup. After all, but most importantly future trials will have to look at combined morbidity and mortality endpoints.

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*Addess for correspondence:*
Prof. Dr. H. Drexler
Abt. Kardiologie und Angiologie
Medizinische Hochschule
Carl-Neubergstrasse 1
D-30625 Hannover, Germany
Tel.: ++49-511-532-3841
Fax.: ++49-511-532-5412
E-mail: drexler.helmut@mh-hannover.de