Myocardial regeneration induced by granulocyte-colony-stimulating factor mobilization of stem cells in patients with acute or chronic ischaemic heart disease: a non-invasive alternative for clinical stem cell therapy?

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Mobilization of stem cells into the peripheral circulation for myocardial regeneration using subcutaneous injections of granulocyte-colony-stimulating factor (G-CSF) has been tested in both patients with acute myocardial infarction (AMI) and patients with chronic myocardial ischaemia. G-CSF treatment seems to be safe and unblinded trials in patients with AMI were encouraging. However, larger double-blind placebo-controlled trials have not been able to demonstrate effect of G-CSF treatment. In patients with chronic myocardial ischaemia, small-unblinded G-CSF trials did not show effect on myocardial perfusion and function. In both patient populations, G-CSF did mobilize stem cells of known importance to myocardial regeneration, but there seemed to be a general lack of homing of the stem cells into the ischaemic myocardium. In AMI, factors of importance to homing of stem cells, stem cell derived factor-1, are maximally elevated in plasma 3 weeks after infarction, suggesting that this time point could be the optimal time for stem cell mobilization treatment. The known complex interaction of stem cells and cytokines for induction of vasculogenesis should be implemented in future clinical trials, to elucidate whether G-CSF mobilization of stem cells might be useful as a new regenerative treatment in patients with ischaemic heart disease.

KEYWORDS

Stem cells; Angiogenesis; Homing; Coronary artery disease; Myocardial infarction; Ischaemia

Introduction

Treatment with bone marrow derived stem cells to regenerate damaged myocardium in patients seems promising. Two different stem cell treatment modalities have been evaluated in clinical trials. Patients were either treated with intracoronary alternatively direct intramyocardial-delivered bone marrow stem cell solutions1–4 or treated with prolonged pharmacological stem cell mobilization from the bone marrow into the peripheral circulation.

Pharmacological mobilization of bone marrow stem cells with granulocyte-colony-stimulating factor (G-CSF) is well known from clinical haematology.5 The majority of stem cells mobilized is CD34+. However, also mesenchymal stem cells (CD34–CD45−) are mobilized.6–15 Because of the non-invasive nature of G-CSF stem cell treatment, and to the fact that a prolonged stem cell response can be obtained, this concept is particularly interesting. G-CSF has been used in several clinical trials in patients with acute myocardial infarction (AMI) and in patients with chronic myocardial ischaemia.6–15 The hypothesis is that the acute or chronic ischaemic myocardium produces and secretes factors, which attract stem cells to the myocardium, and that increased stem cell exposure will increase the regenerative processes and improve myocardial function and perfusion.

On the basis of published clinical studies, this review focuses on the possible future role of G-CSF in the regenerative treatment of both acute and chronic myocardial ischaemia, on the limitations of published clinical trials, and on the safety issues in these trials. We reviewed the peer-reviewed publications identified through searches of MEDLINE using the following keywords: G-CSF, cytokine, stem cells, ischaemic heart disease, coronary artery disease, congestive heart failure, AMI, and primary percutaneous coronary intervention (PCI). Only clinical trials were selected and results from unpublished or ongoing trials were omitted. Publications were cross-checked with relevant references.

Cardioprotective effects of G-CSF in animals

G-CSF is a potent haematopoietic cytokine that influences the proliferation, survival, maturation, and the functional
activation of granulocytes, and it is involved in mobilization of granulocytes, stem, and progenitor cells from the bone marrow into the blood circulation.\textsuperscript{16} The process of mobilization is not fully understood but seems to be mediated through binding of G-CSF to a specific cell-surface receptor, the G-CSF receptor, leading to a subsequent digestion of adhesion molecules by enzyme release, and through trophic chemokines. Stem cell derived factor-1 (SDF-1) and its receptor CXCR-4 seem to play a central role.\textsuperscript{17}

Animal studies have suggested a beneficial effect of G-CSF mobilization of stem cells on left ventricular function after myocardial infarction, with regeneration of myocardium by inducing myogenesis and vasculogenesis and by diminishing post-infarction remodelling.\textsuperscript{18,19} Orič et al.\textsuperscript{18} injected mice with recombinant rat stem cell factor and recombinant human G-CSF to mobilize stem cells for 5 days, then ligated the coronary artery, and continued the treatment with stem cell factor and G-CSF for 3 days. Afterwards, the ejection fraction progressively improved significantly as a consequence of the formation of new myocytes with arterioles and capillaries. These results were confirmed in a recent experiment with G-CSF after reperfused myocardial infarction in rabbits showed improvement in left ventricular ejection fraction and reduced remodelling.\textsuperscript{20} In contrast, G-CSF failed to improve myocardial function in a rat myocardial infarction model after non-reperfused myocardial infarction.\textsuperscript{21} A recent study in mice has indicated that the mechanism by which G-CSF prevents cardiac remodelling after ST-elevation myocardial infarction (STEMI) is by inhibiting apoptosis of cardiomyocytes through the Jak2-STAT3 pathway, rather than through mobilization of bone marrow cells.\textsuperscript{22} In addition, in a murine model of infarction with G-CSF-induced improvement in left ventricular function, an enhanced arteriogenesis and an increase in ICAM-1 expression on endothelial cells were observed.\textsuperscript{23}

### G-CSF treatment in patients with AMI

All published clinical studies of G-CSF treatment after STEMI are summarized in Table 1. The initial small clinical phase 1 trial with G-CSF treatment after AMI treated with PCI indicated that G-CSF treatment improved left ventricular function (Table 1).\textsuperscript{\textsuperscript{7–11}} Kuethe et al.\textsuperscript{8} treated 14 patients with G-CSF 2 days after primary PCI and included a control group of nine patients who refused G-CSF treatment. The G-CSF group had a non-significantly higher increase in ejection fraction at the follow-up when compared with the control group (.78 vs. 3.2%). Almost identical results were found in 20 patients in a single-blinded, placebo-controlled study with G-CSF treatment ~1.5 days after STEMI.\textsuperscript{9} There was a non-significant trend towards an improvement in ejection fraction.\textsuperscript{9} In the larger FIRSTLINE-AMI trial, the 6 months follow-up of 50 patients\textsuperscript{10} and the 1-year follow-up of 30 patients\textsuperscript{11} have recently been published. The trial was a phase 1 randomized but open-label trial of G-CSF treatment initiated within 90 min after primary PCI treated STEMI. The control group did not receive placebo injections, but had identical follow-up. The G-CSF-treated patients had a significant improvement in left ventricular function with enhanced systolic wall thickening in the infarct zone and an improvement in ejection fraction (Table 1). In contrast, the control group had less systolic wall thickening and a decrease in ejection fraction.\textsuperscript{10}

Three double-blind randomized placebo-controlled G-CSF trials could not confirm the uncontrolled phase 1 trial results (Table 1).\textsuperscript{13–15} A total of 78 patients were treated with primary PCI and G-CSF in the STEM cells in Myocardial Infarction (STEMMI) trial.\textsuperscript{13} The results showed no additional effect of G-CSF bone marrow stem cell mobilization on left ventricular myocardial function or infarct size after primary PCI treatment of acute STEMI. The improvements in the STEMMI G-CSF group were comparable to the results of the G-CSF-treated patients in the previous non-blinded phase 1 studies. The important difference between studies is seen in the placebo/control groups. In the STEMMI trial, the placebo-treated patients had the same improvement in cardiac parameters as seen in the G-CSF-treated groups. All MRI data in the STEMMI trial were analysed by a blinded, independent core laboratory, and the demonstrated changes in global left ventricular function in the placebo group were comparable to MRI data of improvements seen in studies of cardiac function after primary PCI.\textsuperscript{24,25} Baks et al.\textsuperscript{24} found an increase in ejection fraction of 7% 5 months after primary stent PCI of acute STEMI. Stone et al.\textsuperscript{25} demonstrated in their study of 2082 patients, with independent core laboratory analysed angiography, a 4–5% increase in ejection fraction after PCI-treated STEMI. In opposition, in uncontrolled trials of G-CSF treatment after primary PCI, the control groups had almost no improvement or deterioration in cardiac function at follow-up compared with baseline measurement (Table 1).

The double-blind, placebo-controlled REVIVAL-2 G-CSF trial\textsuperscript{14} was designed almost identical to the STEMMI trial, except that baseline studies were performed later and that G-CSF treatment was initiated later: 5 days after the primary PCI. In the REVIVAL-2 study, there was no improvement in ejection fraction in the G-CSF group when compared with placebo group (Table 1). The primary endpoint of the REVIVAL-2 trial was change in infarct size from baseline to follow-up. This was, according to scintigraphy, reduced identically in the G-CSF group and the placebo group (Table 1). In agreement, the STEMMI trial did not demonstrate any effect of G-CSF treatment on change in infarct size.

The effect of mobilization of stem cells in patients with subacute STEMI and late revascularization between 6 h and 7 days after onset of angina was investigated in the double-blind, placebo-controlled G-CSF-STEMI trial.\textsuperscript{15} At 3 months follow-up, improvement in ejection fraction was identical in G-CSF and placebo group (Table 1).

The three randomized placebo controlled trials (STEMMI, REVIVAL-2, and G-CSF-STEMI trials) did not confirm the promising results from the small uncontrolled phase 1 clinical trials, despite comparable numbers of circulating stem cells were available for regeneration of the necrotic myocardium with the G-CSF dose used. A G-CSF dose-dependent mobilization rate of CD34+ and mesenchymal stem cells in patients with atherosclerotic heart disease has been demonstrated (Wang et al., submitted for publication), and in most of the trials, a 10–20-fold mobilization of stem cells from the bone marrow into the peripheral circulation has been seen (Table 1).

An important issue might be a mismatch between the activation of homing factors in the necrotic myocardium and the circulating stem cells and hereby an in-optimal timing of the G-CSF treatment. The homing factor SDF-1 is thought to play
<table>
<thead>
<tr>
<th>Study</th>
<th>Design</th>
<th>Cytokine dose and duration</th>
<th>Peak leucocytes level ($10^6$/mL)</th>
<th>Peak CD34+ level ($10^3$/mL)</th>
<th>Follow-up (months)</th>
<th>Endpoint evaluation</th>
<th>n</th>
<th>Baseline ejection fraction</th>
<th>Δ Ejection fraction</th>
<th>Δ End-diastolic volume (mL)</th>
<th>Δ End-systolic volume (mL)</th>
<th>Δ Systolic wall thickening in infarct area</th>
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<tr>
<td>Kuethe et al.(^8)</td>
<td>No-randomized, open-label</td>
<td>G-CSF 10 μg/kg/day mean 7 days</td>
<td>59</td>
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<td>SPECT</td>
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<td>Suarez de et al.(^7)</td>
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<td>G-CSF 10 μg/kg/day</td>
<td>58</td>
<td>80</td>
<td>3</td>
<td>Left ventricular angiography</td>
<td>G-CSF = 13</td>
<td>40</td>
<td>6.2</td>
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<td>Randomized, open-label</td>
<td>G-CSF 10 μg/kg/day 4 days</td>
<td>NM</td>
<td>NM</td>
<td>6</td>
<td>SPECT</td>
<td>Cell Infusion = 7</td>
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<td>Randomized, single-blinded, placebo-controlled</td>
<td>G-CSF 5 μg/kg/day 4 days</td>
<td>NM</td>
<td>34</td>
<td>6</td>
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<td>G-CSF = 10</td>
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<td>↑</td>
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<td>↑</td>
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<td>Ince et al., (^10)</td>
<td>FIRSTLINE-AMI Randomized, open-label, blinded evaluation</td>
<td>G-CSF 10 μg/kg/day 6 days</td>
<td>55</td>
<td>66</td>
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<td>Echocardiography</td>
<td>G-CSF = 25</td>
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<td>6</td>
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<td>3(^b)</td>
<td>0.82(^c)</td>
</tr>
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<td>STEMMI Randomized, double-blinded, placebo-controlled</td>
<td>G-CSF 10 μg/kg/day 6 days</td>
<td>51</td>
<td>55</td>
<td>6</td>
<td>Magnetic resonance imaging</td>
<td>G-CSF = 39</td>
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<td>REVIVAL-2 Randomized, double-blinded, placebo-controlled</td>
<td>G-CSF 10 μg/kg/day 5 days</td>
<td>48</td>
<td>72</td>
<td>4–6</td>
<td>Magnetic resonance imaging</td>
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<td>2.0</td>
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<tr>
<td>Engelmann et al., (^15)</td>
<td>G-CSF-STEMI trial Randomized, double-blinded, placebo-controlled</td>
<td>G-CSF 10 μg/kg/day 5 days</td>
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<td>NM</td>
<td>3</td>
<td>Magnetic resonance imaging</td>
<td>G-CSF = 23</td>
<td>41</td>
<td>6.2</td>
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<td>5.3</td>
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<td>13</td>
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<td>No-randomized, open-label</td>
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<td>62</td>
<td>6</td>
<td>SPECT, echocardiography</td>
<td>G-CSF = 20</td>
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<td>7.9</td>
<td>↑</td>
<td>6.5</td>
<td>-7.9</td>
</tr>
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\(\uparrow\), increased; \(\rightarrow\), unchanged; NM, not mentioned.

\(^a\)Relative increase.

\(^b\)Left ventricular end-diastolic diameter (mm).

\(^c\)Absolute increase (mm).
a crucial role in induction of stem cell engraftment to ischaemic tissue. It has recently been demonstrated that plasma SDF-1 and the vascular growth factors VEGF-A and FGF increase slowly during the first week after a myocardial infarction and reach a maximum concentration after 3 weeks. Therefore, the G-CSF mobilization of stem cells within the first days after a myocardial infarction might not be optimal due to a low concentration of the homing factor SDF-1 within the myocardium. Wang et al. demonstrated that in human myocardium after short-term acute ischaemia there was no induction of SDF-1 or vascular growth factors genes in human. Therefore, a more optimal time point for G-CSF stem cell therapy to induce vasculogenesis in ischaemic/necrotic myocardium might be 1–3 weeks after a STEMI.

In the FIRSTLINE-AMI trial, G-CSF was administered within 90 min after the PCI-treated infarction in opposition to 1–2 days in the STEMMI and 5 days in the REVIVAL-2 trial. However, almost identical improvements were seen in the G-CSF groups in the FIRSTLINE-AMI and the STEMMI trial, whereas the major difference was in the placebo groups, contradicting the hypothesis that a time difference of about 30 h should cause major differences in cardiac regeneration (Table 1).

Different methods have been used to measure a variety of cardiac endpoints in the clinical trials. Because of the high accuracy and reproducibility of cardiac MRI, this method has been suggested as a 'gold standard' for angiogenesis trials. In patients with regional myocardial dysfunction, the regional systolic wall thickening is probably the best parameter to evaluate a G-CSF-treatment-induced change, compared to measurement of the complex parameter of global left ventricular function, which is used in many trial.

In conclusion, treatment with primary PCI after STEMI in most studies results in a significant recovery of the left ventricular function. Additional treatment with mobilization of bone marrow stem cells by subcutaneous injections of G-CSF starting within 5 days after primary PCI do not induce further regeneration of the ventricular function. The discrepancy in results between the initial open-label and the double-blind placebo-controlled STEMMI and REVIVAL-2 trials once again emphasizes the need for blinding and placebo controls in the evaluation of new stem cell therapies within cardiology. Further studies addressing the optimal time of G-CSF treatment and the need for combining G-CSF with factors that increase stem cell homing remains to be considered.

**G-CSF treatment in patients with severe and chronic ischaemic heart disease**

Two small clinical studies have evaluated the safety and clinical effects of stimulation with G-CSF to induce myocardial vasculogenesis in patients with chronic myocardial ischaemia due to stable severe occlusive coronary artery disease (Table 2). In addition, two trials have evaluated the concept of treating patients with stable severe occlusive coronary artery disease by obtaining stem cells from the peripheral blood by apheresis and then inject/infuse these stem cells into the myocardium.

Hill et al. treated 16 patients with coronary artery disease with G-CSF (10 μg/kg body weight) for 5 days...
VEGF-A gene transfer followed by G-CSF mobilization further suggests that a combination of treatment with VEGF-A165 gene transfer followed by bone marrow stimulation to the trial by Wang et al. have prospectively treated 13 patients with severe occlusive coronary artery disease with G-CSF 5 μg/kg body weight for 6 days and included 16 patients as a control group. SPECT demonstrated unchanged number of segments with perfusion defects at rest and stress from baseline to 2 months follow-up in both groups. Blood perfusion during stress was unchanged in the ischaemic myocardium from baseline to follow-up measured by MRI. It was surprising that left ventricle ejection fraction decreased 5% in the G-CSF group from baseline to follow-up measured with MRI and 4% with SPECT and 3% in the control group (ns). However, the ejection fraction was unchanged in both groups by echocardiographic evaluation. This finding could indicate an adverse effect of G-CSF on the myocardium, maybe by an inflammatory response in the microcirculation by the mobilized leucocytes and subsequent development of myocardial fibrosis.

A marked improvement in angina score and reduction in the need for nitroglycerine at the 2 months follow-up visit was seen after G-CSF treatment. These effects were limited to the nine patients with a pronounced mobilization into the peripheral circulating of CD34+ and CD34+/133+ stem cells. At 1-month follow-up, there was no change in left ventricular ejection fraction, in resting or dobutamine stressed left ventricular wall motion or perfusion measured by MRI, nor in treadmill exercise test. Wang et al. have prospectively treated 13 patients with severe occlusive coronary artery disease with G-CSF 5 μg/kg body weight for 6 days and included 16 patients as a control group. SPECT demonstrated unchanged number of segments with perfusion defects at rest and stress from baseline to 2 months follow-up in both groups. Blood perfusion during stress was unchanged in the ischaemic myocardium from baseline to follow-up measured by MRI. It was surprising that left ventricle ejection fraction decreased 5% in the G-CSF group from baseline to follow-up measured with MRI and 4% with SPECT and 3% in the control group (ns). However, the ejection fraction was unchanged in both groups by echocardiographic evaluation. This finding could indicate an adverse effect of G-CSF on the myocardium, maybe by an inflammatory response in the microcirculation by the mobilized leucocytes and subsequent development of myocardial fibrosis.

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On this background, Ripa et al. performed a clinical study to evaluate the safety and clinical effect of VEGF-A165 gene transfer followed by bone marrow stimulation with G-CSF in patients with severe occlusive coronary artery disease. A total of 16 patients were treated with direct intramyocardial injections of the VEGF-A165 plasmid followed 1 week later by subcutaneous injection of 10 μg/kg body weight G-CSF for 6 days. Two control groups were included in the study: 16 patients treated with VEGF gene transfer alone and 16 patients treated with placebo gene injections. The combined treatment with VEGF-A165 gene and G-CSF did not seemed to induce vasculogenesis and did not improve the symptoms in patients with chronic myocardial ischaemia. However, the treatment was safe, and in opposition to the trial by Wang et al., there was no deterioration in left ventricular ejection fraction after G-CSF treatment neither with MRI nor with SPECT.

The lack of clinical effect after mobilization of stem cell with G-CSF cannot be due to a low concentration of circulating stem cells, since all studies demonstrated high levels of putative stem cells in the peripheral blood. However, there seems to be a lack of engraftment of the cells into the chronic ischaemic myocardium. This could be due to identical levels of the homing factor SDF-1 in chronic ischaemic compared to normally perfused myocardium in patients with chronic coronary artery disease. Moreover, we have found identical levels of plasma SDF-1 in patients with chronic ischaemic heart disease and healthy control subjects.

In conclusion, although animal studies have been promising, the clinical safety and efficacy trials with G-CSF bone marrow mobilized stem cells in patients with stable chronic myocardial ischaemia have not been able to show convincing clinical benefit of the treatment. The treatment should possibly be combined with SDF-1 gene therapy to increase engraftment of circulating stem cells into the ischaemic myocardium.

Safety of treatment with G-CSF

In all trials, the subcutaneous injections of G-CSF were in general well tolerated. A few patients experienced mild musculoskeletal pain.

Safety data from a total of 167 patients treated with G-CSF early after STEMI have been published. Two (1.2%) of these patients died in the follow-up period 8,14 one patient (0.6%) had a spleen rupture 7 and one (0.6%) patient had a subacute in-stent thrombosis.12,13

One trial37 has indicated that G-CSF treatment might increase the progression of atherosclerosis and in-stent restenosis in patients treated with primary PCI, maybe by increased inflammation or blood viscosity. In this study, the peripheral circulating stem cells were collected after 3 days G-CSF treatment and then injected into the coronary artery during the PCI procedure. In a recent unblinded uncontrolled study, G-CSF treatment was initiated 2 days after primary PCI and the collected stem cells injected 4 days later into the infarct-related coronary artery.38 They observed a very high restenosis rate at follow-up. However, at the time of stem cell injection into the infarct related artery, one vessel was occluded, four patients needed additional stents to restore normal antegrade flow, one patient had a guide wire induced dissection of the vessel, and only four patients were treated with drug-eluted stents. Therefore, it is more likely that the very high restenosis rate (40%) was procedure and stent-related and not related to the G-CSF treatment. In opposition, both the STEMMI trial,13,39 the FIRSTLINE-AMI trial,10,11 and the REVIVAL-2 trial14 demonstrated identical restenose rates in G-CSF-treated and control groups. This discrepancy in restenosis rates could be that Kang et al. performed the PCI procedure when the G-CSF induced increase in leucocytes was at its maximum, which could increase the angiogenic and inflammatory responses in the culprit lesion.

The inflammatory response to G-CSF treatment as determined by C-reactive protein and erythrocyte sedimentation rates increased slightly in patients with acute and chronic coronary artery disease, but the blood viscosity was unaltered despite the leucocytosis in the FIRSTLINE-AMI trial.10 Moreover, the clinical trials of patients with AMI found no evidence of clinically important worsening of myocardial inflammation in the patients treated with G-CSF.8–15 Still, it cannot be totally excluded that G-CSF may have contributed to the serious adverse events reported from the trials.

It can be speculated whether patients with multi-vessel chronic ischaemic heart disease would be more susceptible to the increases in leukocyte numbers and inflammation via plaque destabilization or growth. Indeed, serious
vascular adverse events has been reported in two patients (13%) by Hill et al., and one patient (20%) by Boyle et al., whereas we have treated a total of 29 patients, without any serious vascular adverse events. It is important to notice that it is a very sick patient population and these few and small trials do not permit us to draw any meaningful conclusions regarding the safety of G-CSF treatment to chronic ischaemic heart disease.

Intramyocardial transplantation of skeletal myoblasts has been shown to induce ventricular tachyarrhythmia in patients. None of the clinical trials with G-CSF treatment to ischaemic heart disease has indicated a pro-arrhythmic effect and a recent experiment in mice has even indicated a reduced inducibility of ventricular arrhythmias after G-CSF treatment when compared with controls.

In conclusion, the treatment with G-CSF seemed to be safe in patients with AMI. In stable chronic myocardial ischaemia, clarification of safety needs studies of more patients and longer follow-up, and preferably the inclusion of a control group.

Summary
Short-term cell mobilization with G-CSF seems safe in patients with AMI, whereas more information is needed in patients with chronic coronary artery disease. In patients with myocardial infarction, most studies showed a significant recovery of the left ventricular function after primary PCI. No convincing additional effect of G-CSF treatment to this recovery of the left ventricular function or perfusion has been shown. One explanation to the lack of effect of G-CSF in these clinical situations might be the lack of a significant myocardial SDF-1 homing signal immediately after AMI and in the chronic ischaemic myocardium. If plasma SDF-1 is an appropriate marker of SDF-1 production in infarcted myocardium, it would probably be better to postpone the G-CSF treatment until 3 weeks after the acute infarction, where SDF-1 spontaneously has increased, or to combine G-CSF treatment with SDF-1 treatment. Also, in patients with chronic coronary artery disease, a combined SDF-1 gene and G-CSF therapy should be considered. The current results do not exclude the possibility that G-CSF, in the future, might be an effective part of a treatment strategy combining several cytokines and/or local stem cell delivery, but presently there is no indication for the use of G-CSF mobilized stem cells for clinical regenerative treatment of infarcted or degenerated ischaemic myocardium.

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