Granulocyte-Colony Stimulating Factor Therapy to Induce Neovascularization in Ischemic Heart Disease

Rasmus Sejersten Ripa

This review has been accepted as a thesis together with 7 previously published papers by University of Copenhagen August 1st 2011 and defended on November 30th 2011

Official opponents: Erling Falk and Hans Erik Bøtker

Correspondence: Department of Cardiology, Rigshospitalet, Blegdamsvej 9, DK-2100 Copenhagen Ø, Denmark

E-mail: ripa@dadlnet.dk

Dan Med J 2012;59(3):B4411

This dissertation is based on the following original publications. These publications are referred to by their roman numerals in the text:

I. Ripa RS, Wang Y, Jørgensen E, Johnsen HE, Hesse B, Kastrup J. Intramyocardial Injection of Vascular Endothelial Growth Factor-A_{165} Plasmid Followed by Granulocyte-Colony Stimulating Factor to Induce Angiogenesis in Patients with Severe Chronic Ischemic Heart Disease. Eur Heart J 2006;27:1785-92.

II. Ripa RS, Wang Y, Goetze JP, Jørgensen E, Johnsen HE, Tägil K, Hesse B, Kastrup J. Circulating Angiogenic Cytokines and Stem Cells in Patients with Severe Chronic Ischemic Heart Disease – Indicators of Myocardial Ischemic Burden? Int J Cardiol 2007;120:181-187.

III. Ripa RS, Jørgensen E, Baldazzi F, Frikke-Schmidt R, Wang Y, Tybjærg-Hansen A, Kastrup J. The Influence of Genotype on Vascular Endothelial Growth Factor and Regulation of Myocardial Collateral Blood Flow in Patients with Acute and Chronic Coronary Heart Disease. Scand J Clin Lab Invest 2009;69:722-728.

IV. Ripa RS, Jørgensen E, Wang Y, Thune JJ, Nilsson JC, Søndergaard L, Johnsen HE, Køber L, Grande P, Kastrup J. Stem Cell Mobilization Induced by Subcutaneous Granulocyte-Colony Stimulating Factor to Improve Cardiac Regeneration After Acute ST-Elevation Myocardial Infarction. Result of the Double-Blind Randomized Placebo Controlled STEMMI Trial. Circulation 2006;113:1983-1992.

V. Ripa RS, Haack-Sørensen M, Wang Y, Jørgensen E, Mortensen S, Bindslev L, Friis T, Kastrup J. Bone Marrow-Derived Mesenchymal Cell Mobilization by Granulocyte-Colony Stimulating Factor after Acute Myocardial Infarction: Results from the Stem Cells in Myocardial Infarction (STEMMI) Trial. Circulation 2007;116(11 Suppl):I24-30.

VI. Ripa RS, Nilsson JC, Wang Y, Søndergaard L, Jørgensen E, Kastrup J. Short- and Long-Term Changes in Myocardial Function, Morphology, Edema and Infarct Mass Following ST-Segment Elevation Myocardial Infarction Evaluated by Serial Magnetic Resonance Imaging. Am Heart J 2007;154:929-36.

VII. Lyngbæk S, Ripa RS, Haack-Sørensen M, Cortsen A, Kragh L, Andersen CB, Jørgensen E, Kjær A, Kastrup J, Hesse B. Serial in vivo Imaging of the Porcine Heart After Percutaneous, Intramyocardially Injected ^{111}Indium-Labeled Human Mesenchymal Stromal Cells. Int J Cardiovasc Imaging 2010; 26:273-84.

1. BACKGROUND AND AIM

The concept of adult stem cells within the bone marrow was introduced in 1960 by identification of cells capable of reconstituting hematopoiesis in mice. (1) Asahara et al (2,3) extended this concept almost 40 years later by showing that bone marrow-derived circulating endothelial progenitor cells incorporated into sites of angiogenesis in animal models of ischemia. In 2001, Orlic et al (4) published a ground-breaking but also very controversial (5,6) trial challenging the paradigm of the heart as a post-mitotic organ thereby igniting the notion of cardiac regeneration. In the following decade an increasing number of animal and small clinical studies have indicated an effect of cell based therapies for ischemic heart disease.

ISCHEMIC HEART DISEASE

Ischemic heart disease is caused by a pathological mismatch between the supply to and demand for oxygen in the left ventricle. The pathology is most commonly stenotic or obstructive atherosclerotic disease of the epicardial coronary artery. The normal coronary circulation supplies the heart with sufficient oxygen to prevent underperfusion. This is accomplished by the ability of the coronary vasculary bed to rapid adaptation of the coronary blood flow by varying its resistance.

An atherosclerotic stenosis increases epicardial resistance and thus limits appropriate increases in perfusion when the demand for oxygen is augmented (e.g. during exercise). In severe stenosis, small changes in luminal diameter (e.g. by spasm or thrombi) can produce significant hemodynamic effects and even reduce myocardial perfusion at rest. Myocardial ischemia can also occur with normal oxygen supply if myocardial demands are markedly increased by left ventricular hypertrophy or during exercise.

The symptoms of myocardial ischemia range from silent ischemia to stable angina pectoris to unstable angina pectoris to non-ST- and ST-segment elevation myocardial infarction (STEMI).

Patients with STEMI or patients with moderate to severe but stable angina pectoris (Canadian Cardiovascular Society (CCS)

angina class II-IV) have been included into the majority of trials with gene- or cell-therapy. The pathology in these two populations has many similarities but also some important differences.

First, patients with STEMI (usually patients with previous myocardial infarction are excluded) have a single, or occasionally a few, severe ischemic events caused by one coronary occlusion whereas the patients with chronic ischemia suffer from intermittent myocardial ischemia (often through years) usually caused by stenotic lesions in several coronary arteries. Second, patients with chronic ischemia typically have reversible ischemia not leading to myocardial necrosis, whereas patients with STEMI develop irreversible myocardial damage. We thus have difference in the therapeutic goals in the two patient populations. Patients with STEMI need new myocytes and vascular support for both new myocytes and for hibernating myocytes within the necrotic area, whereas patients with chronic ischemia primarily need improved perfusion of the reversible ischemic area. This also affects the endpoint assessment in the two populations. Patients with chronic ischemia will be expected to have no change or even a slow deterioration in heart function with their current anti-ischemic treatment whereas patients after STEMI are expected to have a recovery in function due to recovery of hibernating myocardium following balloon angioplasty and coronary stenting. A significant placebo effect can be expected in both populations underscoring the need for a proper control group.

Many early phase clinical trials of new therapies for patients with ischemic heart disease have safety as primary endpoint and efficacy as secondary exploratory endpoint. These early trials are often without control-groups or with non-blinded, non-placebo treated controls. This warrants for extreme caution in data interpretation since both a significant placebo effect as well as a significant change due to 'the natural course' must be accounted for.

BIOLOGICAL INTERVENTION IN MYOCARDIAL REGENERATIVE MEDICINE

Based on our pathogenetic understanding, previous trials of biological intervention in myocardial regeneration can roughly be divided into three main groups, vascular growth factor proteins, genes encoding vascular growth factors, and stem/progenitor cell therapy. Only a few trials have combined these modalities.

Protein therapy

The trials hypothesized that increased supply of vascular growth factors increases neovascularization and thus improve symptoms. The primary goals of the trials were to develop an administration strategy that provided optimal local tissue concentration for an optimal period of time without high systemic concentrations.

The list of known vascular growth factors with angiogenic potential is long and includes vascular endothelial growth factor (VEGF) A,B,C,D,E; fibroblast growth factor (FGF) 1,2,4,5; angiopoitin 1,2; hepatocyte growth factor, monocyte chemotactic protein 1, platelet derived growth factor BB, e-nitric-oxide synthase, i-nitric-oxide synthase, and many more. (7) So far, mainly VEGF-A (8-10) and FGF (11-18) have been used in human trials since these seem to be most important in adult vessel growth.

The VIVA trial (9) and the FIRST trial (14) were the two largest randomized trials using VEGF-A_{165} and FGF-2, respectively. Despite encouraging earlier trials with fewer patients and often without controls both the VIVA trial and the FIRST trial were neutral without any improvement beyond placebo. The explanations for these disappointing results could be several, first VEGF-A and FGF might not have any significant clinical effect, second dose and route of administration may be insufficient in achieving optimal concentration of the growth factor within the heart. The second hypothesis is supported by the short half-life of the administered protein, but administration of a higher dose was not possible due to dose-limiting toxicities resulting from systemic exposure.

Trials with growth factor gene therapy were then initiated to enhance myocardial expression for a sustained period of time and to minimize systemic effects.

Gene therapy

Gene therapy is introduction of genetic material into an organism in order to obtain a therapeutic result by production of proteins. The advantages over protein therapy are primarily less systemic concentrations and prolonged period of expression. Some of the pitfalls are to achieve optimal tissue expression and to prevent expression in other tissues. The gene needs a transfection vector to get into the cells; this can be viruses, liposome particles or naked plasmids. (19) Naked plasmid is the most simple to use, but also a method with low transfection rate.

Several minor safety and efficacy trials using both the VEGF-A and the FGF genes have been published. (20-25) Naked plasmid, liposomes, and viruses have been used as transfection vector, and both intracoronary and intramyocardial (during thoracotomy or percutaneously) administration has been used.

The REVASC Trial (26) randomized 67 patients with severe angina pectoris and coronary artery disease to intramyocardial AdVEGF-A_{121} gene transfer (N=32) or continued maximum medical therapy (N=35). The treatment was open-label, and the control group did not receive placebo treatment. The primary endpoint of change in time to ST-segment depression on exercise ECG after 12 weeks was not statistically significant compared to controls. Several secondary endpoints including exercise test at 26 weeks, and CCS angina class did reach a statistical significant difference. (26)

Our group initiated a multicenter, randomized, double-blind, and placebo controlled trial of plasmid VEGF-A_{165} gene therapy in patients with stable severe angina pectoris (The Euroinject One Trial). (27-29) Intramyocardial injections of the plasmids or placebo were given via the left ventricular cavity using a catheter-based guiding and injection system (the NOGA-Myostar system). Eighty patients with severe stable ischemic heart disease and significant reversible perfusion defects assessed by single photon emission tomography (SPECT) were included. The prespecified primary end point was improvement in myocardial perfusion defects at the 3-months follow-up SPECT and patients were followed with clinical examinations, SPECT, NOGA, exercise test, angiography and echocardiography. Disappointingly, the VEGF-A gene transfer did not significantly improve the stress-induced myocardial perfusion abnormalities compared with placebo. However, local wall motion disturbances (secondary endpoints) improved assessed both by NOGA ($p = 0.04$) and contrast ventriculography ($p = 0.03$). Finally, no gene-related adverse events were observed. (27)

The next step from protein/gene therapy to cell therapy was promoted by these rather discouraging clinical results with protein/gene treatment, and very positive preclinical studies utilizing bone marrow-derived stem- or progenitor cells.

Stem cell therapy

The rigorous definition of a stem cell requires that it possesses self-renewal and unlimited potency. Potency (differentiation

potential) is divided into totipotent (differentiate into embryonic and extraembryonic cell types), pluripotent (differentiate into cells derived from any of the three germ layers), multipotent (produce only cells of a closely related family of cells), and unipotent (can produce only one cell type); strictly only totipotent and pluripotent cells are stem cells whereas multipotent or unipotent cells with self-renewal capacity should be referred to as progenitor cells. It is a matter of ongoing and hectic debate whether committed hematopoietic progenitor cells can undergo transdifferentiation into cardiac myocytes or not. (4-6,30)

Human studies have indicated that mobilization of progenitor and stem cells is a natural response to myocardial injury (31-33) correlating to endogenous concentration of granulocyte-colony stimulating factor (G-CSF). (34) The degree of mobilization seems to predict the occurrence of cardiovascular events and death. (35)

Animal studies showed that bone marrow-derived endothelial precursor cells could induce new blood vessel formation (vasculogenesis) and proliferation from existing vessels (angiogenesis) after myocardial infarction. (4,36) After a quick translation from bench to bedside, several small human safety trials have been conducted in patients with both chronic myocardial ischemia (37-43) and acute myocardial infarction (44-49). Five larger trials with intracoronary infusion of bone marrow-derived mononuclear cells after acute myocardial infarction have been published with diverging results. (50-54) The Norwegian ASTAMI trial (n=100) (51), the Polish REGENT trial (n=200) (53), and the Dutch HEBE trial (n=200) (54) were randomized, but without placebo treatment in the control-arm, whereas the German REPAIR-AMI (n=204) (50) and a Belgian trial (n=67) (52) were both randomized, double-blind, and placebo-controlled. Only REPAIR-AMI showed a significant improvement in the primary endpoint ejection fraction in the active arm (48.3±9.2% to 53.8±10.2%) compared to the control arm (46.9±10.4% to 49.9±13.0%; p=0.02). The trial was not designed to detect differences in cardiac events, but the prespecified secondary combined endpoint of death, recurrence of myocardial infarction, or revascularization at one year follow-up was significantly reduced in the cell group compared with the placebo group (p=0.009). (55) In addition, there was a trend towards improvement of individual clinical endpoint such as death, recurrence of myocardial infarction, and rehospitalization for heart failure. (55) The 2-year follow-up of the REPAIR-AMI trial demonstrated a sustained reduction in major cardiovascular events. In a subgroup of 59 patients magnetic resonance imaging (MRI) showed a higher regional left ventricular contractility and a non-significant difference in ejection fraction. (56) In comparison, 18 months follow-up data from the randomized BOOST trial indicate that a single dose of intracoronary bone marrow cells does not provide long term improvement in left ventricular function when compared to controls. (57) The REPAIR-AMI Doppler Substudy (n=58) has provided insight into the mechanism of intracoronary cells infusions by measuring a substantial improvement in minimal vascular resistance during adenosine infusion 4 months after treatment indicating an improved microvascular circulation. (58)

The hitherto largest published trial of intramyocardial bone marrow-derived cell injection for chronic myocardial ischemia included 50 patients into a double-blind, placebo-controlled trial. (59) The authors reported a significant improvement in stress score by SPECT 3 months after treatment (treatment effect of -2.44 points, p<0.001).

The designs of the trials have so far often been driven by pragmatic solutions, and while some questions have been answered many more have been raised. This has opened for a reverse translation from bedside to bench in order to clarify some of the unknown factors such as optimal cell type and number, optimal route of administration, optimal time of therapy, optimal patient selection, usefulness of repeated or combined treatments etc. (60)

The use of pharmacological mobilization of stem and progenitor cells from the bone marrow into the blood is an attractive alternative to intracoronary or intramyocardial injection because the treatment is noninvasive and does not require ex-vivo purification of the cells. G-CSF is an appealing candidate since it is well known from clinical hematology and thus has an established safety profile. (61)

GRANULOCYTE-COLONY STIMULATING FACTOR

Endogenous G-CSF is a potent hematopoietic cytokine which is produced and released by monocytes, fibroblasts, and endothelial cells. G-CSF regulates the production of neutrophils within the bone marrow and stimulates neutrophil progenitor proliferation, maturation, and functional activation. G-CSF binds to the G-CSF cell surface receptor expressed on myeloid progenitor cells, myeloid leukemia cells, leukemic cell lines, mature neutrophils, platelets, monocytes, and some lymphoid cell lines. (62) Ligand binding induces activation of a variety of intracellular signaling cascades ultimately affecting gene transcription, cell survival and differentiation. (62,63)

G-CSF is involved in mobilization of granulocytes, stem, and progenitor cells from the bone marrow into the blood circulation. (64) The process of mobilization has mainly been investigated for hematopoietic stem and progenitor cells and is not fully understood, but seems to be mediated through binding of G-CSF to the G-CSF receptor, leading to a subsequent digestion of adhesion molecules by enzyme release from myeloid cells, and through trophic chemokines. Stem cell derived factor-1 (SDF-1, also named CXCL12) and its receptor CXCR4 seem to play a central role in regulation of hematopoietic stem cell trafficking in the bone marrow and in mobilization by G-CSF. SDF-1 is a potent chemo attractant for hematopoietic stem cells produced in the bone marrow by stromal cells (65) and its receptor CXCR4 is expressed on the surface of hematopoietic stem cells (66).

SDF-1 protein concentrations in the bone marrow decline sharply during G-CSF treatment. (67) SDF-1 mRNA expression decreases during G-CSF mobilization, and the magnitude of the decline correlates well with the magnitude of mobilization. (68) Studies of CXCR4 deficient mice have shown that this gene is necessary for sufficient retention of myeloid precursors in the bone marrow (69) and neutralizing CXCR4 or SDF-1 antibodies significantly reduced stem cell mobilization. (67) In addition, inhibition of SDF-1 binding to CXCR4 (by AMD3100) leads to rapid mobilization of hematopoietic cells ($CD34^+$) from the bone marrow. (70) The opposite effects of AMD-3100 and neutralizing CXCR4 antibodies are puzzling and could reflect differences in the binding properties of the two molecules.

Several other adhesion molecules are known to regulate hematopoietic stem cell trafficking, such as VCAM-1/β-1 integrin, hyaluronic acid/CD44, kit/kit ligand, and several selectins. (71) G-CSF induces through an unknown mechanism, a proteolytic microenvironment in the bone marrow by release of a number of proteases including neutrophil elastase, cathepsin G, and matrix metalloproteinase 9. (72) These proteolytic enzymes are capable of cleaving the key adhesion molecules within the bone marrow, SDF-1, VCAM-1, and kit ligand. (67,73,74) However, neutrophil elastase, cathepsin G or matrix metalloproteinase 9 deficient mice have normal G-CSF induced mobilization, (75) and thus the

precise mechanism for G-CSF induced cell mobilization remains to be determined.

It has recently been shown that the G-CSF receptor is expressed in cardiomyocytes and that G-CSF activates signaling molecules in cardiomyocytes and hydrogen peroxide-induced apoptosis was significantly reduced by pre-treatment of cardiomyocytes with G-CSF. (76) These results suggest that G-CSF has direct anti-apoptotic effect in cardiomyocytes besides mobilization, differentiation and proliferation of stem or progenitor cells. The proposed molecular mechanisms of these G-CSF induced cardioprotective effects in the subacute-chronic phase are through the Janus kinase 2 / Signal transducer and activator of transcription 3 (Jak2/STAT3) pathway activated by the G-CSF receptor. (76) STAT3 is a transcriptional factor known to activate numerous growth factors and cytokines and has been shown to protect the heart during stress (e.g. in patients with myocardial infarction and during treatment with cytotoxics). (77,78) The cardioprotective effects of G-CSF on post-myocardial infarction hearts were abolished in mice overexpressing dominant-negative mutant STAT3 protein in the cardiomyocytes. (76)

Also recently, G-CSF has been proposed to have an acute "postconditioning-like" effect on the reperfusion injury. (79) G-CSF administration started at onset of reperfusion in a Langendorff-perfused rat heart led to myocardial activation of the Akt/endothelial nitric oxide synthase pathway leading to increased nitric oxide production and ultimately to reduction in infarct size. (79)

Finally, G-CSF has been reported to be an anti-inflammatory immunomodulator by inhibition of main inflammatory mediators such as interleukin-1, tumor necrosis factor-alpha, and interferon gamma. (80,81) Thus, G-CSF could attenuate left ventricular remodeling following acute myocardial infarction by a direct anti-inflammatory effect.

It remains to be determined whether the beneficial effect of G-CSF on cardiac function in animal studies is primarily caused via cell recruitment (82) or via a more direct effect on the myocardium. (76)

Filgrastim is a recombinant methionyl human granulocyte colony-stimulating factor (r-metHuG-CSF) of 175 amino acids. Neupogen is the Amgen Inc. trademark for filgrastim produced by *Escherichia coli* (*E coli*) bacteria. The protein has an amino acid sequence identical to the natural sequence, but the product is nonglycosylated because Neupogen is produced in *E coli*, and thus differs from G-CSF isolated from human cells.

Filgrastim has been used to mobilize hematopoietic stem cells from the bone marrow to the peripheral circulation for the treatment of patients with hematologic diseases for several years, thus Filgrastim treatment has been proven safe and effective in both hematological patients and healthy donors. (83,84) Mild side effects are very frequent (typically bone pain, myalgia, artralgia or headache) but they almost never leads to discontinuation of treatment. Rare side effects (0.01-0.1%) are interstitial pneumonitis, respiratory distress syndrome, thrombocytopenia and reversible elevations in uric acid. Very rare side effects (<0.01%) are spleen rupture and allergic reactions.

The current clinical indications of Filgrastim in Denmark are to (1) reduce the duration of neutropenia in patients with nonmyeloid malignancies undergoing myeloablative chemotherapy followed by marrow transplantation, (2) reduce time to neutrophil recovery following chemotherapy, (3) mobilize stem cells to the peripheral blood, (4) for chronic administration to reduce the incidence and duration of sequelae of severe neutropenia.

PATHOGENESIS OF MYOCARDIAL REGENERATION

This section gives a short review of the mechanisms and variables of importance for clinical biological intervention. It is focused on vascular regeneration and the impact of cellular components, growth factor and cytokines.

Embryonic development and subsequent postnatal adaptation of the vascular system to chances in functional needs occur by three different processes: (1) vasculogenesis, (2) angiogenesis, or (3) arteriogenesis (review in (85)). This nomenclature is not always strictly followed, and some even uses the term 'angiogenesis' to summarize all types of vascular formation. All tree processes involves a cascade of different cell types, numerous soluble and cell-bound factors, transcription factors, and cell receptor expression in a complex coordinated interaction that is still not completely described. The below description is an overview of the processes and some of the most important steps involved. The mechanism of how bone marrow-derived cells influence neovascularization remains debated (page 7): Do the cells incorporate into the tissue (e.g. as endothelial or smooth muscle cells) or do they primarily act through paracrine signaling to support the vessel growth and/or maturation?

Vasculogenesis is the first process in embryonic vascular development and denotes an in situ differentiation of endothelial precursor cells (hemangioblasts (86)) into blood vessels. The mesoderm-derived angioblasts migrate into clusters (blood islands) and mature into endothelial cells that assemble into a primitive vascular network in both the yolk sac and the embryo (review in (87)) The process is regulated by a cascade of growth and transcriptional factors, proteases and receptor expressions. The initiating signal for vasculogenesis in embryology is probably tissue ischemia due to rapid tissue growth. CXCR4 and SDF-1 are expressed during embryonic development (88) and a role in angioblasts migration to ischemic areas could be assumed. FGF-2 and VEGF-A appear paramount in subsequent blood island formation, cell differentiation and vascular maturation. (89-91)

Tissue ischemia and exogenous granulocyte macrophage-colony stimulating factor (GM-CSF) or VEGF-A has been shown, in animal studies, to stimulate postnatal vasculogenesis by mobilization and differentiation of endothelial precursor cells. (92,93) Like angiogenesis, the process of postnatal vasculogenesis within ischemic tissue is driven by hypoxia-induced production of cytokines and growth factors like VEGF-A (94) and SDF-1 (95). Postnatal vasculogenesis requires extravasation and migration of the progenitor cells as described on page 13.

Angiogenesis is the capillary growth (sprouting) from existing vessels. The term also involves division of existing vessels by transendothelial cell bridges or pillars of periendothelial cells. Angiogenesis is initiated by hypoxic stabilization of the transcription factor hypoxia-inducible factor (HIF)-1α. (96) This leads to a local upregulation in expression of VEGF-A and a number of other angiogenic factors. (97) The new sprouting vessel is initiated in one endothelial cell lining the native vessel (the 'tip cell'). The endothelial cell exposed to the highest VEGF-A concentration is selected as the endothelial tip cell. (98,99) Furthermore, this tip cell seems to gain competitive advantage over neighboring endothelial cells by VEGF-A induced upregulation of 'delta-like 4'. Delta-like 4 activates Notch receptors on the neighboring cells leading to a down-regulation of delta-like 4 expression in these cells. (100) VEGF-A exerts its effect in angiogenesis primarily through binding to the VEGFR2. (99) The tip cell becomes a polarized non- or low-proliferative cell with filopodia extending to-

wards and 'sensing' the angiogenic stimuli and environment. The sprout elongates by migration of the tip cell and proliferation of endothelial 'stalk cell' trailing behind the tip cell. (98,99) The stalk cells form junctions from the tip cell to the native vessel and form a lumen in the new sprout. The migration of the tip cell is an invasive process requiring proteolytic degradation of the extracellular matrix, especially the 'membrane type-1 matrix metalloproteinase' appears paramount for the invasion. (101) Eventually the sprout connects with another sprout by tip cell fusion. (102) The new tubular structure is stabilized into a mature vessel by tightening of cellular junctions, recruitment of pericytes and deposition of extracellular matrix. Normoxia of the tissue once the new vessel is perfused lowers the local VEGF-A concentration leading to quiescent of the endothelial cells (named 'phalanx cells') and vascular homeostasis.

Arteriogenesis denotes the formation of muscular arterioles from preexisting capillaries or small arterioles. Postnatal arteriogenesis is widely studied in collateral vessel circulation following arterial occlusion. The temporal sequence of arteriogenesis is divided into the initiation phase, the growth phase, and the maturation phase.

In contrast to angiogenesis, arteriogenesis seems initiated by physical forces experienced by the cell independent of ischemia. A pre-existing network of small caliber collateral anastomoses exists in humans. Arterial occlusion (e.g. by atherosclerotic plaque) result in a drop in pressure distal to the occlusion. This new pressure gradient across the occlusion drives the flow along the smaller pre-existing bridging arteries to circumvent the occlusion. Increased flow in the collateral arteries creates a shear stress and circumferential tension at the wall sensed by the smooth muscle cells and endothelial cells. The physical stimuli in the smooth muscle cells seem to increase expression of the proarteriogenic molecule, 'monocyte chemotactic protein-1' via the mechanosensitive transcription factor 'activator protein-1'. (103) The mechanical stimuli of endothelial cells modulates endothelial gene expression (104) and gene expression analysis following hindlimb ischemia in mice have identified differential expression of more than 700 genes. (105) Very fast surface expression of adhesion molecules on the endothelial cells (106) as well as expression of inflammatory cytokines (105) leads to recruitment of bone marrow-derived cells and differentiation of collateral artery smooth muscle cells to a synthetic phenotype. (106) The next 'growth' phase of arteriogenesis result in luminal expansion. This is accomplished by a degradation of the basal membrane, (106,107) and outward migration and proliferation of the vascular cells triggered by a number of signaling pathways involving both growth factors (108) and paracrine signaling from recruited bone marrow-derived cells. (109) As luminal diameter increases, shear stress decreases, and expression of inflammatory cytokines decreases. (105) In this 'maturation' phase, collateral vessels can either mature and stabilize or undergo neointimal hyperplasia and regression. The fate of the vessel is probably determined by the hemodynamic forces, that is, the largest and most developed vessels will stabilize and the smaller and less developed vessels will regress. (110)

A number of cell populations from the bone marrow play a role in arteriogenesis. These participate in a temporally coordinated process in the different phases of arteriogenesis. Neutrophil leukocytes are the first cells to infiltrate the vessel during the initial phase (within a few hours) through binding to the adhesion molecules expressed by the endothelial cells, but the neutrophils are only present in the first few days of the process. (111) The neutrophils seem to recruite inflammatory monocytes to the growing vessel (112) perhaps mediated by VEGF-A release. The monocyte has a paramount role in arteriogenesis (113) and accumulates in the vessel shortly after the neutrophil recruitment (106) that is, in the growth phase of arteriogenesis. Depletion of macrophages seems to eliminate flow-induced remodeling of the vessel in mice. (114) The origin of the inflammatory cells involved in arteriogenesis remains controversial. An experiment in rats could indicate that inflammatory leukocytes and monocytes/macrophages at least in the first days of the process comes from proliferation of tissue resident cells rather than from the circulation. (115)

Bone marrow-derived stem- and progenitor cells

This paragraph aims to give a brief overview of the bone marrow-derived cells potentially involved in cardiac cell-based therapy. Three cell populations from the bone marrow are typically described in cardiac cell based therapies: the hematopoietic stem/progenitor cells, the endothelial progenitor cells (EPC), and the multipotent mesenchymal stromal cells (MSC). Irrespective of the cell type, several potential mechanisms of cell based therapies can be hypothesized. These mechanisms can be both direct by incorporation and differentiation of the cells into cardiac or/and vascular cells, or indirect by secretion of paracrine factors, cytoprotection, or immunomodulatory effects (page 7). The main source of progenitor cells is thought to be the bone marrow, but cells from other tissues like fat most likely also contribute. (116,117) A number of resident cardiac stem/progenitor cell has been identified and also appear involved in cardiac myogenesis (review in (118)). These cells will not be described further in this overview.

Hematopoietic stem/progenitor cells is multipotent cells that can differentiate into all the blood cell types, both in the myeloid and the lymphoid cell lineage and has unlimited capacity of self-renewal. Numerous studies of bone marrow transplantation in hematological patients have documented the possibility of restoration of bone marrow and hematopoietic function (119); however the precise phenotype and characteristic of the hematopoietic stem cells remain debated.

Hematopoietic stem cells have been isolated from bone marrow and peripheral blood as cells expressing CD34 and/or CD133. The number of cells expressing CD34 predicts hematopoietic recovery after blood stem cell transplantation (120) and are thus used to assess the numbers of peripheral blood hematopoietic progenitor/stem cells in the clinic.

The interest in myocyte-differentiation potential of the hematopoietic stem cells was motivated by the still controversial publication in Nature by P. Anversas group. (4) The authors found that transplantation of hematopoietic stem cells into infarcted mice hearts led to myocardial regeneration apparently through transdifferentiation of hematopoietic stem cells to functional myocytes. These results were later reproduced by the same group (30,121), whereas other groups could not. (5,6,122)

Endothelial progenitor cells are found in the bone marrow and in peripheral blood. There has been and is a continued debate over the phenotype and functional characteristics of EPC.

The term EPC has typically been cells in the blood or the bone marrow co-expressing a hematopoietic (CD34, CD133) and endothelial markers (e.g. VEGFR, CD31, Tie-2). However, this phenotype is not exclusive to EPC. Another approach to EPC isolation is to plate peripheral blood mononuclear cells to give rise to colo-

nies. This result in two cell populations: the 'early outgrowth EPC' (also called proangiogenic haematopoietic cells) and the extremely rare 'late outgrowth EPC' (also called endothelial colony-forming cells). (123) The late outgrowth cells have rapid proliferation and seem to include true stem/progenitor cells. They are reported to have a $CD34^{+}CD45^{-}$ phenotype and express VEGFR2 but not CD133 or CD14. (124) The majority of published studies of EPC have used early outgrowth EPC.

The number of circulating EPC following acute myocardial ischemia increases (31,125) whereas patients with 3-vessel disease undergoing diagnostic cardiac catheterization have low numbers of circulating EPC. (126) Several drugs used in patients with myocardial ischemia increases the concentration of EPC in the blood e.g. ACE-inhibitors and statins. (127,128)

Circulating putative EPC were first isolated by Asahara et al. (3) who cultured cells expressing CD34 or VEGFR2. The cells differentiated into a endothelial-like phenotype and incorporated into areas with vasculogenesis/ angiogenesis where the cells appeared integrated into the capillary wall. (3) Shi et al. found in a similar study that a subset of $CD34^{+}$ cells could differentiate into endothelial cells in vitro in the presence of FGF, insulin-like growth factor 1, and VEGF-A. (129)

The mechanism of EPC contribution to adult angiogenesis and arteriogenesis is not clarified but the prevailing belief is a paracrine rather than a direct incorporation and differentiation of the cells (page 7). This is supported by their capability of releasing angiogenic growth factors including VEGF-A, SDF-1, and insulin-like growth factor 1. (130)

Transdifferentiation of EPC into cardiomyocytes has been reported by the group of S. Dimmeler, (131,132) however, like in the case of hematopoietic stem cells these results have been difficult to reproduce by others. (133)

Multipotent Mesenchymal stromal cells: Nearly 40 years ago Friedenstien et al. described that fibroblast-like (stromal) cells from the bone marrow were capable of reconstituting the hematopoietic microenvironment at ectopic sites. (134) Later, research identified the multipotent bone marrow stromal cells (MSC) that can differentiate into mesodermal cell lines. The group of Verfaillie has even described a *pluripotent* cell-type (termed multipotent adult progenitor cells (MAPC)) purified from the bone marrow. (135,136) Noteworthy though, evidence for pluripotency of MAPC has been difficult to reproduce by others.

MSC is often isolated from the bone marrow, but has been identified in a number of tissues, including fetal and umbilical blood, lung, liver, kidney and adipose tissue. (137) It has recently been shown that pericytes (138) (cells surrounding epithelial cells in capillaries and microvessels) and cells residing in the tunica adventitia (139) share antigenic markers and behave similarly to MSC in culture. It has thus been proposed that the natural MSC niche is perivascular both within bone marrow and other tissues. (139)

Both the defining characteristics and the isolation procedure of MSC differ among investigators due to a lack of simple sensitive and specific markers. MSC is often isolated by plastic adherence and a fibroblastic appearance. Flow cytometry is an easy approach for cell phenotyping based on cell-surface antigens. Unfortunately, no sensitive and specific marker-set of MSC has been found – in contrary a huge list of markers expressed or not-expressed by MSC isolated by different groups from different tissues exist (140) making comparisons of published results difficult. In addition, often MSC phenotypes are described after in vitro culture and little is known about the in vivo phenotype.

To complex matters more, the nomenclature is ambiguous. Terms like colony forming units fibroblasts, mesenchymal stem cells, marrow stromal cells, mesenchymal progenitor cells), mesodermal progenitor cells, skeletal stem cells, multipotent mononuclear stem cell, non-hematopoietic stem cell, and multipotent adult progenitor cell probably name the same cell population (at least to some extent).

The International Society for Cellular Therapy recommended in 2005/2006 'multipotent mesenchymal stromal cell' (MSC) as the designation for plastic-adherent cells isolated from bone marrow and other tissues. The following three minimal criteria for defining MSC were suggested: (1) plastic-adherent when maintained in standard culture conditions, (2) Specific surface phenotype (must express CD105, CD73, CD90 and must lack expression of CD45, CD34, CD14 or CD11b, CD79α or CD19, HLA-DR), and (3) In vitro differentiation into osteoblasts, adipocytes and chondroblasts. (141,142)

MSC has been shown to differentiate into both endothelial cells (143,144), vascular smooth muscle cells (145) and cardiomyocytes (146,147). However, another study indicate that MSC cannot acquire a mature cardiomyocyte phenotype. (148) MSC has been shown to express anti-apoptotic, angio- and arteriogenic factors like interleukin 6, VEGF-A, leukemia inhibitory factor, and matrix metalloproteinase 2. (149) Enzyme-linked immunosorbent assay of MSC medium contained secreted VEGF-A, insulin-like growth factor 1, hepatocyte growth factor, adrenomedullin, placental growth factor and interleukin 6. (149,150) These characteristics of MSC could indicate both a potential direct (by cell engraftment and differentiation) and indirect (by paracrine) effect.

MSC are reported to express a number of functional chemokine receptors (151) allowing for their migration in response to chemokine gradients in damaged tissue. However, some controversy exist e.g. over the expression of the CXCR4 receptor. (152) Myokardial infarctions, bone fractures, and renal injury are examples where transplanted MSC has been shown to home to the damages area. (153-155) Passage of the endothelial barrier is essential for tissue homing of circulating cells. MSCs has been shown in vitro to interact by P-selectin and VCAM-1/β1-integrin with endothelial cells under shear flow, thus allowing egress from the bloodstream. (156) The SDF-1/CXCR4 signaling axis is a strong candidate for MSC migration (155,157,158) although one recent study could not show an effect of CXCR4 inhibition on MSC migration to ischemic tissue (159) and another study indicate that 'monocyte chemotactic protein 3' is an important MSC homing factor. (160)

Numerous studies have described a positive effect of MSC therapy on ischemic tissue (e.g. increased capillary density in infracted area (161) or reduce scar formation after myocardial infarction. (162-164)). The majority of engraftment studies show, that only a small fraction of intravenous MSC engraft, and of these, only a small fraction differentiates. (165) A growing number of studies support the hypothesis that the benefit of MSC transplantation comes from release of paracrine molecules. (109,166) These effects could potentially be angiogenic, anti-apoptotic, anti-inflammatory or perhaps through a paracrine effect on resident cardiac stem cells.

Peripheral blood multipotent mesenchymal stromal cell (PBMSC): The existence of MSC in the peripheral blood under homeostatic conditions remains controversial. It is also unclear where PBMSC originates and where they go. As with bone marrow-derived MSC, terminology and isolation procedures differ among investigators

(review in (167)), this may contribute to the mixed results regarding PBMSC. PBMSC are often isolated as adherent, clonogenic, and fibroblast-like and thus also termed colony-forming units-fibroblastic (CFU-F). CFU-F from peripheral blood (typically following G-CSF treatment) has been claimed identified by several groups. (168-170) The frequency of CFU-F from peripheral blood varies widely among studies but is low (or even absent (171,172)) compared to the frequency in bone marrow-derived mononuclear cells. (167) A trial by Kassis et al. comparing isolation of PBMSC by plastic adherence with fibrin microbeads-based isolation could indicate that a suboptimal isolation procedure enhances the low yield of PBMSC in many trials. (173)

The immunophenotype of CFU-F from peripheral blood share many similarities with bone marrow-derived-MSC but also some differences. They lack CD34, CD45, and HLA-DR and express CD90 and CD106 as bone marrow-derived MSC do. In contrary to marrow-derived MSC, CD133 has been reported expressed (169), and CD105 are not always expressed (168). These differences open the question if PBMSC are bone marrow-derived MSC mobilized to the blood or a distinct cell population.

Potential mechanisms of cell-based therapy

Improved myocardial function after cell based therapies was initially ascribed vascular and/or myocardial regeneration by a direct action of transplanted cells through myogenesis and/or vasculogenesis. Different lines of stem- and progenitor cells were repeatedly demonstrated to differentiate into endothelial cells, vascular smooth muscle cells and myocytes. (4,145,174,175) However, an increasing number of studies have shown a remarkable lack of sustained engraftment and differentiation of the transplanted cells. (165) Another observation is the absent correlation between the number of transplanted cells and functional improvement. These observations have led to the hypothesis that the improved function after cell therapy may – at least in part – be caused by secretion of paracrine factors rather than differentiation. Potential paracrine effects could be neovascularization (potentially vasculogenesis, angiogenesis and arteriogenesis), improved remodeling and contractility as well as myocardial protection and/or cardiac regeneration by resident cells. The importance of neovascularization was confirmed by Yoon et al who demonstrated in a very elegant design that vascular differentiation (endothelial and smooth muscle lineage commitment) of bone marrow-derived mononuclear cells is critical in left ventricular recovery following acute myocardial infarction. (176) Elimination of cardiac-committed cells in the same study did not affect ejection fraction.

A growing body of evidence for the paracrine hypothesis exist (review in (177)). Some of the most notably studies have shown that conditioned medium from stem/progenitor cells can reproduce the functional results observed after cell transplantation. (178-181) Shabbir et al. (182) found in an unusual setup, that injection of MSC into skeletal muscle improved cardiac function although the transplanted cell appeared to be trapped in the skeletal muscle. The authors found evidence that MSC-derived interleukin 6 activated skeletal muscle-cell Jak/STAT3 pathway. Skeletal muscle then increased expression of VEGF-A and hepatocyte growth factor that supposedly had a positive effect on heart failure. This study could indicate a very complex cascade from transplanted cell to target organ involving several cell-types and trophic factors.

The paracrine mechanism opens the opportunity for protein-based rather than cell-based therapy once the paracrine factors are identified. However, the temporal and spatiel co-operation between several beneficial factors could be so complex that a cell-based strategy would still be most optimal.

AIM AND HYPOTHESIS

With this background it has been the *aim* of this translational programme to establish and evaluate cell based therapies using G-CSF as a treatment modality for patients with ischemic heart disease. It has been our hypothesis that clinical effective cardiac regeneration requires cellular components and exogeneous/ endogeneous modulating molecules in symphoni. Therefore, we

- Evaluated safety and effects of combined treatment with G-CSF and VEGF-A-gene therapy in patients with chronic ischemic heart disease.[I]
- Investigated if inherent differences in patients could serve as markers for selecting patients for gene- or cell-therapy.[II,III]
- Evaluated the clinical effect and safety of treatment with G-CSF following STEMI[IV] and reasons for failed effect of G-CSF.[V]
- Determined the recovery in left ventricular function and morphology after current guideline treatment of STEMI.[VI]
- Evaluated a method for intramyocardial in vivo cell tracking.[VII]

This review will aim at presenting the implications and conclusions of our studies in relation to other investigations.

2. TRIAL DESIGN

MEASURES OF EFFICACY

The optimal and conclusive efficacy endpoint in a cardiovascular trial is allcause mortality or perhaps morbidity. However, this would require a huge patient population which is neither ethically nor economically justifiable for neovascularization trials at present. One key issue in our trial designs has thus been to find the best surrogate endpoint available.

For patients with stable chronic ischemia, one approach is the patient's subjective assessment of symptoms and wellbeing since we would expect only minor changes in the disease without new intervention. For some patients with chronic disease this endpoint may be more important than prolongation of life. (183) However, this evaluation of 'quality of life' will require a strict control for the substantial placebo-effect instituted by our invasive treatment and by the close follow-up of our tendering study nurses.

To diminish the significance of influence from the placebo effect, a number of more objective measures of cardiac function and perfusion can be considered.

Myocardial volumes and function

Myocardial function and left ventricular volumes are traditionally assessed using echocardiography, but also ventriculography, SPECT, positron emission tomography (PET), computed tomography (CT), and MRI can be used. (184) Most often, myocardial function is assessed at rest, but it can be visualized during pharmacological or even physiological stress. Change in left ventricular ejection fraction is often used as primary endpoint. This seems reasonable since ejection fraction has been shown to predict mortality. (185) However, ejection fraction at rest can be preserved despite large infarctions due to hypercontractility of non-infarcted myocardium. (186) Regional function may be more informative and the wall motion score index has been found to be superior to ejection fraction in predicting prognosis following myocardial infarction. (187)

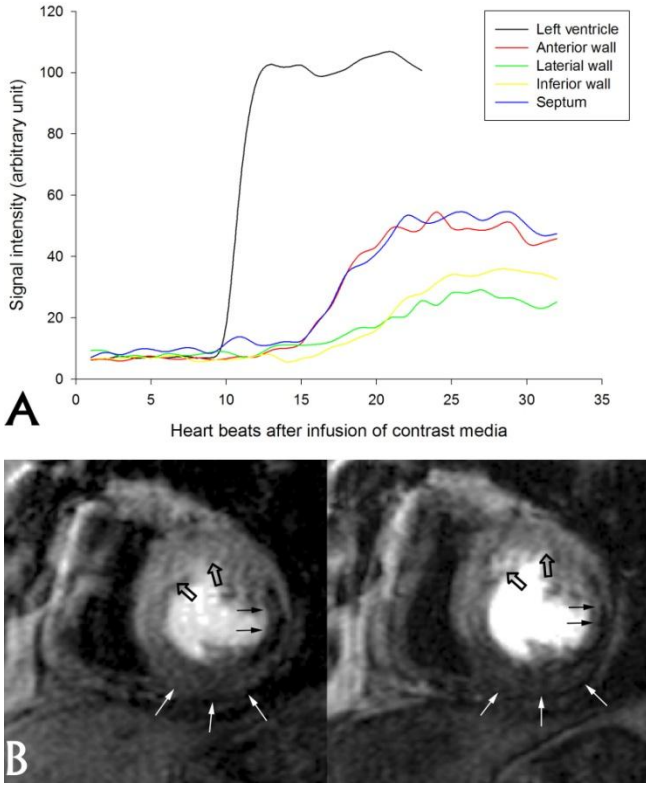

Figure 1
Example of MRI perfusion scan in one patient with infero-lateral ischemia treated with VEGF-A165 gene transfer followed by G-CSF.
A: Time versus signal intensity curves showing the fast initial contrast input into the cavity of the left ventricle and subsequent contrast enhancement in the myocardium.
B: Baseline and follow-up examination. Images from 20 sec after contrast infusion, demonstrating poor perfusion with no contrast enhancement in the lateral wall (black arrows), moderate perfusion with attenuated enhancement (white arrows) in the inferior wall, and normal perfusion in the anterior wall (transparent arrows).[I]

2D echocardiography is widely used in clinical practice and research because it is fast, easily accessible, and contains no radiation exposure but is also dependent on the operator and the acoustic window. In addition, quantification of left ventricular volumes rely on some geometric assumptions that are not always met especially in ischemic cardiomyopathy. (188) These limitations result in an only moderate accuracy (median limits of agreement from ±16 to ±19%) when compared to radionuclide or contrast ventriculography. (189)

ECG gated SPECT allows assessment of left ventricular volumes (190) using an automated 3-D reconstruction of the ventricle and the method has a good reproducibility. (191) The primary drawbacks are the use of ionizing tracers, the long acquisition time and the low temporal resolution. In addition, low spatial resolution limits the assessment of regional wall motion.

At present, most investigators consider MRI as the gold standard for assessing global and regional left ventricular function due to high accuracy and reproducibility combined with high spatial resolution.

The advantages of MRI for functional evaluation compared to other imaging techniques are its non-invasiveness, the use of non-ionizing radiation, independence of geometrical assumptions and acoustical windows, and no need of contrast media. The primary drawbacks are low (but improving) temporal resolution, and low accessibility. The examination of patients with tachycardia, especially irregular, (e.g. atrial fibrillation) or implanted ferromagnetic devices such as implantable cardioverter-defibrillator and pacemaker is problematic or impossible. Furthermore, MRI scanners may cause claustrophobia in many patients. The STEMI trial[IV] included 78 patients and MRI was not feasible in 20 patients (25%) primarily due to claustrophobia. This is more than usually expected, but the patients were psychologically fragile due to the very recent STEMI. Another recent MRI trial early after acute myocardial infarction showed an even higher drop-out rate. (53)

The cinematographic MRI technique used for the measurements of cardiac volumes also poses problems. Image information for each frame in a given position is sampled over a number of consecutive heart cycles (15 in our trials) within a set time-window (50 ms in our trials); the process is termed segmented k-space sampling. This temporal resolution can 1) cause problems in defining the frame with endsystolic phase, 2) cause blurring of the endo- and epicardial borders since the myocardium is contracting in the 50 ms time window, and 3) the required breath-hold during the 15 heart cycles can be difficult for the patients. Furthermore, only one short axis slice could be obtained within a single breath-hold (in end-expiration) with our equipment. Thus, if the point of end-expiration varies from slice to slice, this affects the position of the diaphragm, resulting in non-consecutive slices.

Partial volume effect can be a problem near the base and apex, since each slice has a thickness (8 mm in our trials). This may result in imprecise border definitions.

Despite these problems several studies have reported high accuracy (192-194) and reproducibility (195,196) in determining left ventricular volumes and thus function. Still, echocardiography will fulfill the clinician's needs in the vast majority of cases, whereas MRI is a sophisticated alternative primarily indicated for research purposes.

Myocardial perfusion

Regional myocardial perfusion is another important endpoint in trials of cardiac neovascularization since these therapies are hypothesized to induce capillaries and small arterioles not visible by coronary angiography. Gamma camera imaging and PET have been used for perfusion assessment for more than a decade and more recently CT, contrast echocardiography and MRI (197) have advanced within this field. Perfusion can be visualized during both rest and stress (pharmacological or physical).

SPECT is probably the most available clinical method for perfusion assessment. The myocardial uptake of the radioactive tracers' thallium 201 and technetium 99m labeled sestamibi/tetrofosmin is proportional to the blood flow. The method is limited by high ionizing radiation, low spatial resolution (aprox. 10 mm with our equipment) and frequent image artifacts. In comparison PET has better spatial resolution (6-10 mm) but is still insufficient to detect minor subendocardial defects. With PET absolute perfusion can be quantified by dynamic imaging of radioactive isotopes as they pass through the cardiovascular system. PET is less prone to attenuation artifacts than SPECT since accurate attenuation correction can be done. However, PET is expensive and has low accessibility.

CT and contrast echocardiography is emerging as modalities for perfusion assessments. The great advantage is the high spatial resolution (<1 mm) but more validation and optimization of the methods remains.

MRI can quantify the myocardial perfusion by dynamic imaging of the first pass of a paramagnetic (non-ionizing) contrast agent through the heart (Figure 1). (197-200) The modality has an

acceptable spatial (2-3 mm) and temporal (0.5-1.0 s) resolution, but is not widely validated and it is cumbersome to assess the absolute perfusion using this method. The method is further limited by recent accumulating evidence that MRI contrast media containing gadolinium (especially gadodiamide) can cause irreversible nephrogenic systemic fibrosis in patients with renal insufficiency. (201) To date, nephrogenic systemic fibrosis has only been reported in patients with severe renal impairment.

Conclusion

There are several surrogate endpoints and methods with clinical relevance for neovascularization trials. PET offers accurate measure of perfusion and left ventricular volume during stress and rest with higher spatial resolution than SPECT. Echocardiography is very accessible and has excellent temporal resolution for volume assessment. The MRI technology offers a range of high-quality endpoints with very high spatial resolution within a single examination without a need for radiation. In the design of each trial it remains important to choose the primary endpoint with most clinical relevance.

ETHICAL CONSIDERATIONS IN TRIAL DESIGN

Treatment with gene or cell therapy is a new area of research warranting for caution in study design. The primary concern is and must be the safety of the treatment and the secondary concern is the efficacy of the treatment. This is not different from traditional drug-trials, but this being a new treatment modality should probably demand for an even higher bar of safety than usually required. The real question is how to gain this knowledge or assumption of safety? Ultimately, we need large double-blinded and randomized patient groups followed for a long period of time. Obviously, this is not possible or even ethical with a new treatment modality where we need to base our initial safety assumption in theoretical knowledge of the treatment (what side effects do we expect knowing the potential effect of the treatment?), early animal trials, early phase clinical trials with few (perhaps healthy) individuals, and the gradual increase in patient number if the treatment still seems safe and effective. In the ethical consideration, it is also important to account for the morbidity of the patients before inclusion. Very ill patients with poor prognosis and without any treatment options will probably accept higher risk than patients with a more benign disease.

The trials included in this thesis have primarily focused on treatment with one pharmacon (G-CSF) in patients with either severe chronic myocardial ischemia[I] or following acute myocardial infarction[IV]. G-CSF is a registered drug (page 4) used for years in healthy donors and patients with hematological diseases. The drug is generally well tolerated with few and mild side effects. However, the drug was not formally tested in patients with ischemic heart disease. At the time of the trial design there was increasing evidence from animal and small clinical trials that autologous bone-marrow derived cells (mononuclear cells) led to improved myocardial function via neovascularization and perhaps myogenesis. (2,4,36,42,46,202-204) It was believed that hematopoietic stem or progenitor cells were the 'active substance'. (174,205,206) Mobilization of cells from the bone marrow seemed like an attractive alternative to intracoronary or intramyocardial injection that would not require bone marrow aspiration or cardiac catheterization. G-CSF was known to mobilize hematopoietic cells from the bone marrow into the circulation (page 3). Animal studies had shown that circulating stem and progenitor cells are attracted to ischemic myocardium and incorporates into the formation of new blood vessels. (36,92) On this background Orlic et al (207) 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 as a consequence of the formation of new myocytes with arterioles and capillaries. (207)

Our initial studies with G-CSF included patients with severe chronic ischemic heart disease [(208),I] since we found more evidence that bone marrow derived cells would promote neovascularization, than neogeneration of myocytes. We thus hypothesized that patients with severe chronic ischemia would potentially benefit more from the treatment compared to patients with acute myocardial infarction or heart failure. In addition, the clinical experience with G-CSF to patients with ischemic heart disease was at that time limited. Despite good long term safety results from hematology, we initially included patients only with severe morbidity without any options for further conventional treatment. All patients included went through a strict screening procedure including a renewed evaluation by independent cardiologists and thoracic surgeons to ensure that no conventional treatment was possible.

Later, accumulating evidence (31,37,44,45,209,210) of both safety and efficacy of G-CSF lead us to initiate a trial with G-CSF to patients with STEMI.[IV,] (211)

3. G-CSF FOR CHRONIC ISCHEMIA

Hill et al (212) and our group (208) have treated patients with chronic myocardial ischemia due to stable severe occlusive coronary artery disease with G-CSF to induce myocardial vasculogenesis and angiogenesis. Both trials were small, non-randomized safety trials with few patients (n=16 and n=13). Three other trials have included patients with intractable angina to treatment with G-CSF and subsequent leukopheresis and intracoronary cell infusion. (213-215).

We showed a similar increase in CD34+ cells in the blood following G-CSF treatment. (208) The perfusion defects at rest and stress assessed with SPECT demonstrated unchanged number of segments from baseline to 2 months follow-up. This was confirmed with MRI where myocardial perfusion during pharmacological stress was unchanged in the ischemic myocardium from baseline to follow-up. Left ventricular ejection fraction decreased from baseline to follow-up measured with MRI (from 57±12 to 52±11, p=0.01), and the trend was the same with SPECT (from 48±10 to 44±12, p=0.09), whereas the ejection fraction was unchanged 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.

The change in subjective clinical outcomes were more positive, CCS class improved from 2.7±0.6 to 1.7±0.6 (p=0.01), nitroglycerin consumption from 1.5±2.1 to 0.5±1.2 per day (p<0.05), and number of angina pectoris attacks per day from 1.7±1.7 to 1.0±1.6 (p<0.05). (208) The interpretation of these subjective measures is not easy. On one hand this endpoint is most important for the patient (who does not care about improvement in SPECT); on the other hand this is a non-randomized study with only historical controls making placebo effect a potential confounder. However, the clinical improvement seems restricted to patients with a pronounced mobilization into the peripheral

circulating of $CD34^+$ stem suggesting a causal relationship. (208) It can be speculated if the treatment with G-CSF led to deterioration of perfusion and thus infarction of previously ischemic myocardium, this might explain the deterioration in ejection fraction and the diminished symptoms of ischemia.

G-CSF AND VEGF-A GENE THERAPY

G-CSF therapy increased the vascular supply of bone marrow-derived cells to the myocardium but did not improve myocardial perfusion and function. (208,212) We hypothesized that this could be caused by a lack of signals from the myocardium to engraft the cells into the ischemic myocardium. VEGF-A_{165} has been demonstrated to be of importance for the differentiation of stem cells into endothelial cells participating in the vasculogenesis (93) and is also important in the homing of cells to ischemic areas (page 13). Animal studies further suggest that a combination of treatment with VEGF-A gene transfer followed by G-CSF mobilization of stem cells might be superior to either of the therapies. (216,217)

On this background, we performed a clinical study to evaluate the safety and clinical effect of VEGF-A_{165} gene transfer followed by bone marrow stimulation with G-CSF in patients with severe occlusive coronary artery disease.[I] Sixteen patients were treated with direct intramyocardial injections of the VEGF-A_{165} plasmid followed 1 week later by subcutaneous injection of G-CSF for 6 days. Two historic control groups from the Euroinject trial (27) were included in the study: 16 patients treated with VEGF-A gene transfer alone and 16 patients treated with blinded placebo gene injections. The treatment was well tolerated and seemed safe with no serious adverse events during the combined VEGF-A_{165} gene and G-CSF treatment or in the follow-up period.[I] Also we had no serious procedural events during intramyocardial injection of the VEGF-A_{165} gene in these 16 patients. However, it is known that NOGA mapping and injection is not a risk free procedure. Five patients (6%) in the Euroinject One Trial had serious procedure-related complications, two of these were at our institution. (27) Similar events following the NOGA procedure has been reported by others. (59) It is our impression that most of these events can be avoided with increased experience by the staff. Approximately 100 NOGA procedures have been performed at our institution without any serious events since the two described events (J. Kastrup, personal communication). We have also detected significant (but usually only minor) release of cardiac markers (CKMB and troponin T) following the NOGA procedure. (218)

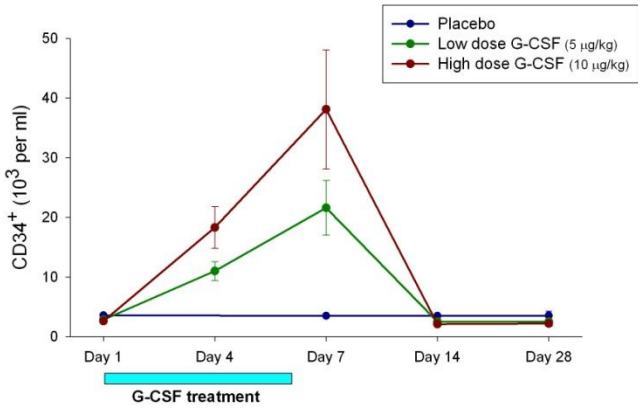

Figure 2
Circulating $CD34^+$ cells from baseline to day 28 after different treatment strategies.[(219)]

The treatments lead to a significant increase in circulating $CD34^+$ cells as expected after the G-CSF treatment (Figure 2). (219,220) The prespecified primary efficacy endpoint of change in perfusion defects at stress SPECT came out neutral after 3 months follow-up (Figure 3), this result was confirmed with MRI measurement of myocardial perfusion during adenosine stress (baseline 62%±32 to 74%±32 at follow-up, p=0.16).[I] In addition, there was no significant difference in changes in CCS classification, angina pectoris attacks, nitroglycerin consumption, or exercise time between the three groups (Figure 4). In opposition to the trial with G-CSF as monotherapy (208), there was no deterioration in resting left ventricular ejection fraction after G-CSF treatment neither with MRI nor with SPECT.[I] This trial has several limitations, and primarily it must be considered if this trial was underpowered to detect a difference especially since we included few patients with short follow-up period into an open-label design with only historical controls. Furthermore, we were unable to analyze all patients for all endpoints due to technical difficulties and in some instances poor image quality. In favour of our results are the facts that multiple endpoints using different methods consistently have shown virtually identical results from baseline to follow-up. In conclusion, we found no indication of clinical effects or improved myocardial function following combined treatment with VEGF-A_{165} gene transfer and G-CSF.

Of interest, a trial (*clinicaltrials.gov*, NCT NCT00747708) is currently being conducted in patients with congestive heart failure secondary to ischemic heart disease. The investigators aim to include 165 patients into several treatment arms to investigate G-CSF alone or in combination with intracoronary/intramyocardial cell injections.

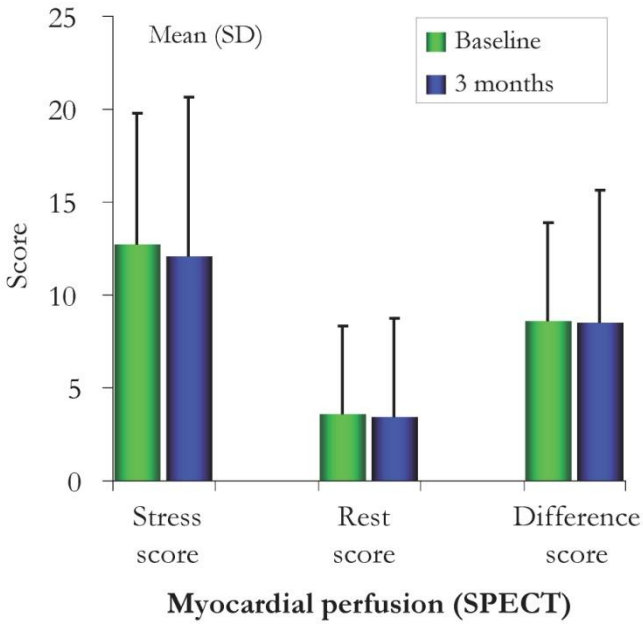

Figure 3
Myocardial perfusion at rest and stress measured by SPECT after treatment with VEGF gene therapy and subsequent bone marrow cell mobilization with G-CSF.[I]

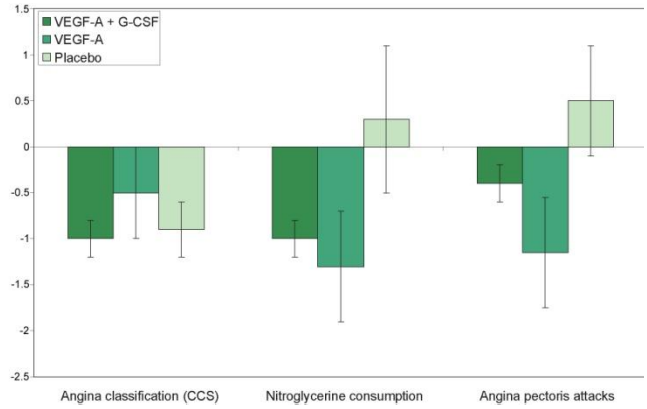

Figure 4
Changes in angina pectoris in patients treated with placebo, plasmid VEGF-A_{165} or plasmid VEGF-A_{165} and G-CSF. [I]

SAFETY OF G-CSF TO PATIENTS WITH CHRONIC ISCHEMIA

Our group has treated a total of 29 patients with severe chronic myocardial ischemia with G-CSF without any serious vascular adverse events. Serious vascular adverse events have been reported in two patients (13%) by Hill et al (212) and one patient (20%) by Boyle et al (213). In a rigorous trial by Kovacic et al (214) 4 patients (20%) had episodes of cardiac ischemia with elevated troponin I and either ECG changes or elevated CKMB, in addition troponins were elevated in 17 other occasions but without ECG changes or elevated CKMB after G-CSF. The patients described the episodes as typical for their usual angina pattern. The authors speculate if these troponin elevations reflect the natural history of refractory angina, or the G-CSF treatment, since the trial was not placebo controlled. (213)

Patients with multi-vessel chronic ischemic heart disease are potentially susceptible to the G-CSF-induced increase in leukocyte numbers and inflammation via plaque destabilization or growth. However, a study of cholesterol fed swine suggests that the administration of G-CSF causes neither exacerbation nor modification of atherosclerotic lesions. (221)

The few and small trials do not permit us to draw any meaningful conclusions regarding the safety of G-CSF treatment to chronic ischemic heart disease. Clarification of safety needs studies of more patients with longer follow-up, and preferably the inclusion of a control group.

DOES G-CSF REDUCE MYOCARDIAL ISCHEMIA?

No convincing effect of G-CSF has been described in patient with chronic ischemia. (208,212)[I] There have been indications of improved subjective measures of efficacy in several trials but objective measures of myocardial perfusion and ischemia were unchanged. We cannot exclude the possibility that the trials have been underpowered and/or endpoint assessment to poor to find a statistical significant difference. The GAIN II trial included 18 patients with chronic ischemic heart disease into a randomized placebo-controlled double-blinded crossover trial of G-CSF using a more accurate primary endpoint (myocardial perfusion by MRI) than SPECT. The trial was presented at ACC 2010 (222), but remains unpublished. The authors found no effect of G-CSF on the primary endpoint.

Several explanations to the apparent lack of effect of G-CSF in this clinical setting can be suggested.

Angiogenesis or arteriogenesis

Our pretrial hypothesis was that G-CSF and VEGF-A gene therapy would increase angiogenesis in reversible ischemic myocardium by engraftment and differentiation of bone marrow-derived cells. We chose G-CSF since it was a known mobilizer of progenitor/stem cells from the bone marrow, and VEGF-A because it was a major contributor in cell homing (page 13) and angiogenesis (page 4). Further, we included patients with at least one open epicardial artery to the ischemic area since angiogenesis without epicardial blood supply will probably be of little effect. Retrospectively, it is plausible that increasing the number of circulating cells and the tissue expression of VEGF-A is simply 'to easy' despite earlier encouraging results. Both vasculogenesis and angiogenesis involve a complex cascade of several cells types and numerous soluble factors – VEGF-A being just one important player.

It should also be considered if arteriogenesis rather that angiogenesis should be the primary aim of neovascularization. Despite an open epicardial artery, blood flow to the capillaries may still be compromised and arteriogenesis would result in large caliber conductance arteries that could more effectively restore blood flow to ischemic myocardium. Recent evidence from a clinical trial of patients with chronic stable coronary artery disease suggest that G-CSF has the capacity to promote coronary collateral growth. (223) Fifty-two patients were randomized to a two week period with G-CSF or placebo every other day. Both ECG signs of ischemia and collateral flow index in a stenotic coronary artery during balloon occlusion improved after G-CSF indicating an improved collateral function.

Patient population

It can be speculated whether angiogenic mechanisms to improve blood supply to the ischemic heart are already activated in patients with severe chronic myocardial ischemia, leaving little therapeutic effect for exogenous angiogenic therapy.

We investigated the plasma concentration of factors known to influence angiogenesis, cell mobilization and homing as well as putative stem/progenitor cells in 54 patients with severe chronic ischemia and 15 healthy controls.[II] Surprisingly, we found that, in general, circulating stem/progenitor cells and plasma concentra-

Table 1
Circulating cell and cytokine concentrations in patients with chronic myocardial ischemia and control subjects

	Patients (n=54)	Control subjects (n=15)	p-value
VEGF-A (10^{-12}*g/ml)	35.0 (18.6-51.4)	91.7 (8.2-175.1)	0.4
FGF-2 (10^{-12}*g/ml)	9.0 (6.2-11.7)	11.3 (2.2-20.3)	0.7
SDF-1 (10^{-10}*g/ml)	22.48 (21.24-23.72)	20.14 (17.50-22.79)	0.2
$CD34^+$ (10^3/ml)	2.8 (2.4-3.3)	3.0 (2.1-3.9)	0.6
$CD45^-/CD34^-$ (10^4/ml)	20.8 (17.0-24.6)	21.9 (13.0-31.0)	0.8

Values are mean (95% confidence interval)

tions of angiogenic related cytokines were not significantly different from the control group (Table 1). This result could be influenced by the fact that plasma concentrations of the cytokines are perhaps a poor indicator of the concentrations within the myocardium. A more precise measurement would require cardiac catheterizations of the patients, which is not clinically applicable for patient stratification, and furthermore the procedure could potentially influence the cytokine concentrations as has been shown for pro-b-type natriuretic peptide. (224) In addition, measuring plasma VEGF-A is potentially influenced by release from the platelets during collection and processing of the blood. We have standardized the procedures to diminish this source of error but cannot exclude that this could explain some of the large inter-patient variations observed. However, our results are supported by our finding of unaffected VEGF-A mRNA contents in chronic ischemic myocardial tissue compared to normally perfused myocardium. (225)

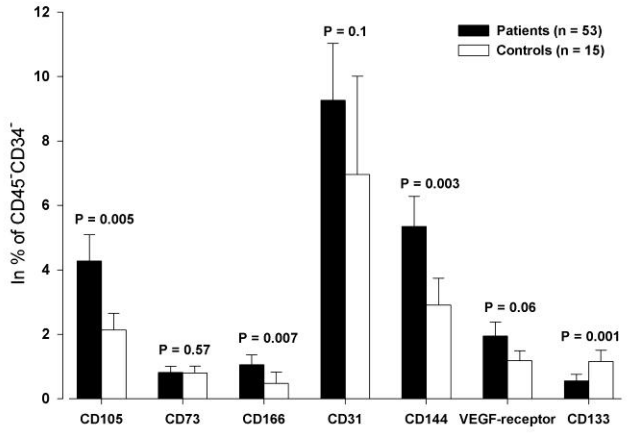

Figure 5
Subclassification of circulating $CD45^-/CD34^-$ cells in patients with ischemic heart disease compared to healthy controls. *(Reprinted from ref. I with permission from Elsevier)*

We assessed the number of peripheral blood MSC in patients and control subjects using flowcytometry identification of circulating mononuclear cells negative for both the endothelial marker CD34 and the pan-leukocyte marker CD45.[II] This identification procedure has low specificity for MSC since we did not include any positive marker (like CD105, CD73 and CD90), thus our nomenclature in the paper was imprecise since the whole population of $CD45^-/CD34^-$ cells should not be referred to as putative stem cells (Page 6). Our reasons for focusing on the population of $CD45^-/CD34^-$ circulating cells were several. First, at the time of analyses of the blood no consensus on which surface markers that determined MSC existed. In contrary, different surface markers on MSC where described in an increasing number of publications (Page 6). Second, very few had published results regarding surface markers identifying PBMSC (168). And third, we had identified cells within a population of $CD45^-/CD34^-$ cells with a mesenchymal-like phenotype after culture. (32) We decided on this basis to focus our attention on the $CD45^-/CD34^-$ cells knowing that this would result in an unspecific identification of MSC. We feared that including positive surface markers in the identification process would exclude some of the MSC, since we could find only little consistency regarding surface markers of PBMSC in the literature. We did include a number of surface-markers in the sub classification of the $CD45^-/CD34^-$ cells. These were chosen from the literature to include both markers often reported to be expressed by bone marrow-derived MSC (CD105, CD73, CD166), (140,226) markers found on endothelial cells (CD31, CD144, VEGFR2) (140) and a marker expressed by hematopoietic progenitor cells that has also been found on PBMSC, but not in bone marrow-derived MSC (CD133) (140,169). We observed that the fraction of $CD45^-/CD34^-$ cells co-expressing surface markers expressed by bone marrow-derived MSC (CD 105 and CD 166) and endothelial cell markers (CD31, CD144, VEGFR) were higher in ischemic patients compared to controls (Figure 5)[II] indicating that ischemia mobilizes both endothelial committed cells and more undifferentiated cell. We found some evidence for a relation between the severity of the ischemia and VEGF-A and FGF-2 concentrations in the patient group. Due to large inter-individual variations of these angiogenic factors as reflected by the inability of the markers to separate healthy from sick, they do not appear to be suitable as markers for selecting individual patients for gene or cell therapy.[II]

We performed another trial to test the hypothesis that germline DNA variations in the VEGF-A promoter and 5´ untranslated region were associated with the plasma concentration of VEGF-A, and that these DNA variations as well as the VEGF-A plasma concentration influence the ability to open coronary collateral arteries in patients with acute and chronic obstructive coronary heart disease.[III] The plasma concentration of VEGF-A was significantly increased in patients with acute coronary heart disease compared to patients with chronic coronary heart disease, but the inter-patient variations were large.[III] This is in concordance with results showing that VEGF-A plasma concentration seems to increase shortly after acute myocardial infarction[IV] and that VEGF-A mRNA expression is low in chronic ischemic myocardium but increased in acute ischemia and reperfused myocardium. (225) A model combining four polymorphic loci (including two in Hardy-Weinberg disequilibrium) in the VEGF-A gene promoter and 5´ untranslated region seemed to explain about 30% of the variation in plasma concentration of VEGF-A, but this model was identified in a stepwise analysis including many loci and should thus be interpreted with caution.[III] Coronary collaterals can be assessed by several methods. We could not perform invasive measurements in these patients and chose to assess collateral flow and function indirectly using two previously described angiographic methods. The Rentrop classification assesses collateral filling of the epicardial artery (227) whereas the Werner classification assesses the size of recruitable collaterals following occlusion of an epicardial artery. The Werner classification has been shown to closely reflect both invasively determined collateral resistance and the collateral functional capacity to preserve ventricular function. (228) We identified an inverse association between the VEGF-A plasma concentration and the size of the collaterals as classified by the Werner classification (228) in patients with chronic myocardial ischemia.[III] The present results could suggest that patients with lower concentration of circulating VEGF-A have decreased coronary collateral function. This is in concordance with other trials showing that polymorphism in the VEGF-A promoter is associated with impaired prognosis in heart failure (229) and affects diseases such as proliferative diabetic retinopathy (230) and end-stage renal disease. (231) It can be speculated if the neutral results of larger clinical trials with VEGF-A treatment (9,27) are affected by differences in the VEGF-A gene leading to a very heterogeneous patient population regarding VEGF-A plasma concentration. It would be of conceptual interest to include analysis of size of coronary collaterals or even hypoxic regulation of VEGF-A by in-vitro assay in future trials of neovascularization for cardiovascular disease.

Homing of bone marrow-derived cells into ischemic myocardium
All studies have demonstrated high concentrations of putative hematopoietic stem or progenitor cells in the blood after treatment with G-CSF (Figure 2). (I,208,212) It can be speculated if these cells home into the chronic ischemic myocardium.

Homing is the process where circulating cells migrate into the target tissue by traversing the endothelial barrier. The general consensus that cell-based therapies primarily derive from paracrine actions requires homing of the cells (regardless of type) to the ischemic area since the secreted factors are limited to a local area with high concentration. Cell homing is in many aspects a mirror process of bone marrow cell mobilization.

Most of the knowledge regarding cell homing and migration comes from studies on hematopoietic stem cells and EPC but it is natural to assume related mechanisms for other cell populations. Local tissue ischemia induces fast increased expression of chemokines, where SDF-1 and VEGF-A in particular has attracted much attention. (232,233) Homing of cells is a multistep procedure involving interaction with the host endothelium, transmigration through the endothelial barrier and migration into the host tissue. Human $CD34^+$ cells initiate the low affinity rolling phase on E- and P-selectin. (234) A high affinity adhesion results from β2- and β1-integrin interaction with their counter ligands expressed on the endothelial cells (ICAM-1 and VCAM-1). SDF-1 expressed on the endothelia appear crucial in this process of integrin adhesion. (234,235) The next step of extravasation involves SDF-1 induced cell polarization (236) and degradation of the basal membrane probably by matrix-degrading enzymes, β2-integrin appear important in this process. (237) The next step of migration towards the ischemic area guided by chemokine gradient also involve proteolytic activity e.g. by cathepsin L. (238)

The main proposed mechanism of G-CSF therapy to ischemic heart disease is mobilization and subsequent homing of progenitor cells to the ischemic area. The myocardial homing of G-CSF mobilized cells in patients with ischemic cardiomyopathy may be impaired by a number of factors: (1) microarray analysis and real-time PCR could not demonstrate any significant difference in SDF-1 expression between chronic ischemic myocardium and normally perfused myocardium. (225) A study of gene-expression in human limbs following amputation similarly showed a low expression of VEGF-A, SDF-1, and CXCR4 in chronic ischemic tissue compared to non-ischemic tissue. (239) This impaired chemokine respons could potentially reduce cell homing substantially. (2) The angiogenic potency of bone marrow cells have been shown reduced in patients with chronic ischemic heart disease as well as a number of conditions related to ischemic heart disease, such as high cholesterol, high c-reactive protein, aging, renal failure, anemia etc. (240,241) The angiogenic potential of bone marrow-derived MSC does not seem impaired in patients with chronic ischemic heart disease. (242) (3) Endothelial dysfunction seems to impair neoangiogenesis by reducing tissue nitric oxide synthase expression. (243-245) (4) The Jak/STAT pathway is a downstream target from CXCR4 on EPC that modulates cell migration. It was recently shown that Jak-2 phosphorylation in response to SDF-1 was reduced in patients with ischemic heart disease indicating a functional impairment of the cells. (246) (5) G-CSF in itself may also affect the bone marrow-derived cells, as impaired migratory capacity following G-CSF has been found. (247,248) Another study showed that G-CSF mobilized bone marrow-derived mononuclear cells do not engraft in chronic ischemic myocardium. Engraftment was only observed after transplantation of SDF-1 expressing fibroblasts. (232) Finally, G-CSF was shown to reduce expression of adhesion molecules involved in the homing process (CXCR4, β2- and β1-integrin) on circulating $CD133^+$ cells in the RIVIVAL-2 trial. (249)

Animal studies have shown that VEGF-A gene transfer combined with G-CSF therapy leads to incorporation of bone marrow-derived cells into ischemic myocardium. (216) However, in animal studies, chronic ischemia will include components of acute and subacute ischemia as well. Most animal studies induce chronic myocardial ischemia, using an ameroid constrictor around the circumflex or anterior descendent artery. Four to five weeks later the myocardium is often called chronic ischemic myocardium. However, the intracellular milieu is probably in many respects not similar to patients' myocardium suffering from repetitive chronic ischemia for several years.

Imaging studies (page 19) are warranted to elucidate the issue regarding homing and engraftment of injected/infused cells in vivo.

4. G-CSF FOR STEMI

CLINICAL TRIALS

Encouraging animal and laboratory results led to a quick translation from the bench to patients suffering an acute myocardial infarction and numerous small sample safety and efficacy trials have been conducted and published. (250-255)

Kuethe et al (251) included 14 patients to subcutaneous G-CSF 2 days after primary percutaneous coronary intervention (PCI) and nine patients who refused G-CSF treatment were included as control group. The authors found a non-significantly larger increase in ejection fraction in the G-CSF treated patients when compared with the control group (7.8 vs. 3.2%). A single-blinded, placebo-controlled study with G-CSF treatment 1.5 days after STEMI (n=20) found almost identical results with a non-significant trend towards improvement in ejection fraction (252). Leone et al (253) randomized 41 patients 1:2 to unblinded G-CSF or conventional treatment. All patients had anterior STEMI and ejection fraction<50 at inclusion. After 5 months there were a significant improvement in left ventricular ejection fraction (p=0.02) and absence of left ventricular dilatation (p=0.04) when compared to conventional treatment. Remarkably, patients receiving conventional treatment had no change in ejection fraction in the follow-up period (from 38±6% to 38±8%). (253) The larger FIRSTLINE-AMI (256) trial (n=50) was a phase 1 randomized, open-label trial of G-CSF treatment initiated within 90 min after primary PCI treated STEMI. The control group did not receive placebo injections. The G-CSF-treated patients had a significant improvement in left ventricular function with enhanced systolic wall thickening in the infarct zone (from 0.3±0.2mm to 1.1±0.3mm) and an improvement in ejection fraction (from 48±4% to 54±8%). In contrast, the control group had less systolic wall thickening (from 0.3±0.3% to 0.6±0.3%) and a decrease in ejection fraction (from 47±5% to 43±5%) measured with echocardiography. (256) The finding that patients in the control group did not experience any improvement in left ventricular ejection fraction is remarkable and not consistent with other clinical studies (page 18) and daily clinical experience.

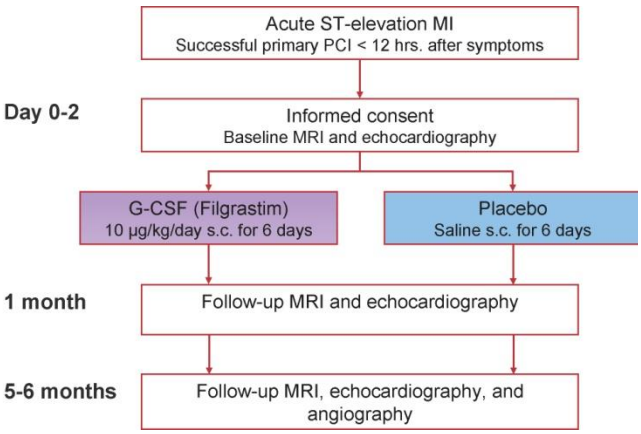

Figure 6
STEMMI trial design

Four randomized, double-blinded, placebo-controlled G-CSF trials have been published and all reached similar conclusions despite some differences in study design.[IV,] (257-259) The REVIVAL-2 (258) and the STEMMI[IV] trials included patients with STEMI treated with PCI within 12 hours after symptom onset (Figure 6). Patients in the STEMMI trial (N=78) received the first G-CSF or placebo injection 10 to 65 hours (with 85% initiated <48 hours after PCI), and five days after the PCI in the REVIVAL-2 trial (N=114). The primary endpoint in the STEMMI trial was change in regional systolic function (systolic wall thickening) and this did not differ significantly between the placebo and G-CSF groups (17±32 versus 17±22 percentage points, Figure 7).[IV] Left ventricular ejection fraction improved similarly in the two groups measured by both MRI (8.5 versus 8.0; P=0.9) and echocardiography (5.7 versus 3.7; P=0.7). The infarct sizes were unchanged in the 2 groups from baseline to the 6-month follow-up.[IV] This was probably due to the small sample size, since pooling of all the patients in the STEMMI trial suggested a significant decrease in infarct mass during the first month[VI] – a result similar to other MRI studies. (260) The STEMMI trial has some inherent limitations that increase the risk of a false negative result. First, we included 78 patients, and 54 (69%) were available for paired analysis of the primary endpoint – this is a small population even though the pretrial analysis indicated a 90% power to detect a significant change.

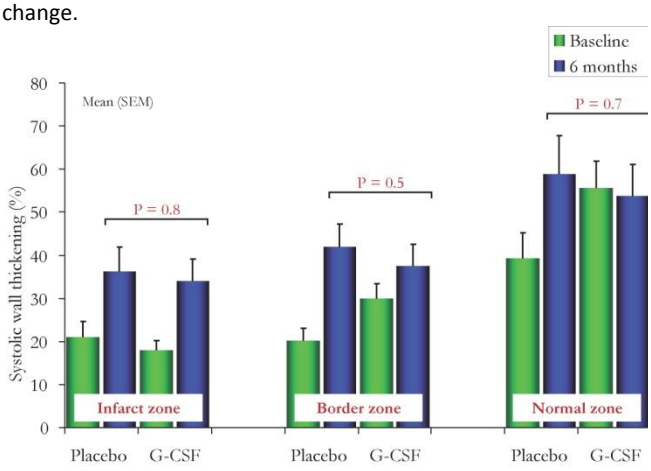

Figure 7
Primary endpoint of regional systolic wall thickening efter G-CSF or placebo treatment.[IV]

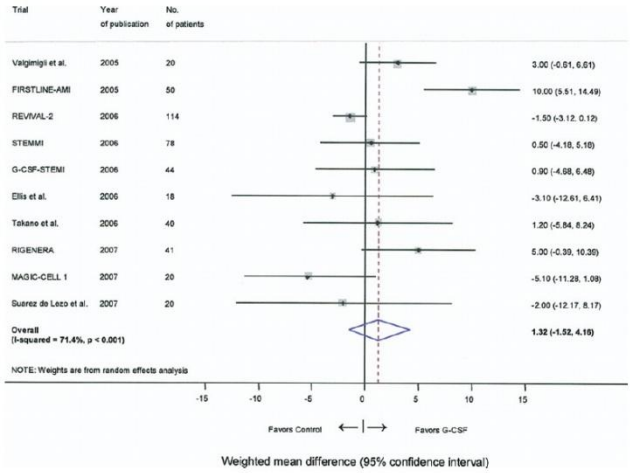

Figure 8
The effect of G-CSF treatment on recovery of ejection fraction – a meta-analysis.
(Reprinted from ref. 261 with permission from Elsevier)

Second, the trial was designed to include a homogeneous population where early MRI was possible (mean ejection fraction 53% and infarct size 13g); this probably led to exclusion of high-risk patients who would potentially benefit most from the treatment. (50) We intended to increase statistical power by using a paired design with an accurate method (MRI). The primary endpoint of the REVIVAL-2 trial was reduction of left ventricular infarct size according to technetium 99m sestamibi scintigraphy. Between baseline and follow-up, left ventricular infarct size was reduced by a mean (SD) of 6.2% (9.1%) in the G-CSF group and 4.9% (8.9%) in the placebo group (P=0.56). Ejection fraction was improved by 0.5% (3.8%) in the G-CSF group and 2.0% (4.9%) in the placebo group (P=0.14).

Engelmann et al (259) included patients (N=44) undergoing late revascularization (6-168 hours) after subacute STEMI. G-CSF was initiated approximately 1½ day after the PCI. Global myocardial function from baseline (1 week after PCI) to 3 months improved in both groups, but G-CSF was not superior to placebo (Δejection fraction 6.2±9.0 vs. 5.3±9.8%, p = 0.77). Ellis et al (257) included few patients (N=18) into a pilot dose-escalation randomized trial and found no effect of G-CSF on left ventricular ejection fraction.

In a recent meta-analysis we aimed to evaluate the effect of cell mobilization by G-CSF on myocardial regeneration after acute myocardial infarction. (261) Ten randomized trials, including 445 patients, were included. Compared with placebo, stem cell mobilization by G-CSF did not enhance the improvement of left ventricular ejection fraction at follow-up (Figure 8, mean difference 1.32% [95% confidence interval -1.52 to 4.16; p = 0.36]) or reduction of infarct size (mean difference -0.15 [95% confidence interval -0.38 to 0.07, p = 0.17]). (261)

SAFETY OF TREATMENT WITH G-CSF AFTER STEMI

A major issue to consider is the possibility that the neutral outcome of the G-CSF trials is the result of undetected adverse outcomes balancing any benefits of the G-CSF treatment.

In all reported trials G-CSF was generally well tolerated. Only a few patients experienced minor musculoskeletal pain, a well known side-effect of G-CSF.

Safety data from more than 200 patients treated with G-CSF early after STEMI have been published. Four of these severely ill patients died in the follow-up period (251,255,258,259), one

patient had a spleen rupture (250) and three patients had a sub-acute in-stent thrombosis or re-infarction.[IV,] (211,257,259) We recently performed a 5-year clinical follow up of the patients included in STEMMI (presented as abstract (262)). The clinical events were combined into 4 prespecified endpoints: Time to first (1) hospital admittance (all cause), (2) cardiovascular related hospital admittance, (3) major cardiovascular event, (4) Death. Survival analyses in this small cohort showed no differences in the occurrence of any of the 4 prespecified composit endpoints between the two groups (p=0.6; 0.5; 0.8; 0.3). This result must be interpreted with extreme caution due to the low number of both patients and events.

Trials by Kang et al (263) and Steinwender et al (264) have indicated that G-CSF treatment increase the progression of atherosclerosis and in-stent restenosis if initiated a few days prior to stent implantation, maybe by increased inflammation or blood viscosity. However, both trials have several potentially inflicting issues. In the trial by Steinwender et al (264) at the time of 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-eluting 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. The trial by Kang et al included only few patients, into a clinically irrelevant design with a very late stent revascularization. (263) The trial by Kang et al was published in Lancet in the middle of the inclusion period of the STEMMI trial. This obviously gave us severe concern regarding the continued inclusion of patients into the trial despite the differences in study design. We chose to do a non-prespecified interim analysis of baseline data, 1-week blood tests, and data from the 5 months invasive follow-up (including intravascular ultrasound) from all patient included at that time (n=41) to evaluate whether it was safe to continue inclusion in the STEMMI trial. (265) The analyses of the intravascular ultrasound and angiograms were performed in a blinded fashion by an independent core laboratory (Bio-Imaging Technologies B.V., Leiden, The Netherlands). In conclusion, we found identical re-stenosis rates in G-CSF-treated and control groups by quantitative coronary angiography and by intravascular ultrasound and which legitimized continued inclusion. (265) We found it most likely that the differences compared to the trial by Kang et al was caused by significant differences in the timing of G-CSF administration in relation to PCI. Later, our preliminary results were confirmed in the total STEMMI population[IV,] (266), the FIRSTLINE-AMI trial (256), the G-CSF-STEMI trial (259), the REVIVAL-2 trial (258), and in a meta-analysis (267). In addition, a recent new report with more patients and longer follow-up from Kang et al do not support their initial conclusions. (254)

Treatment with G-CSF leads to a slight increase in inflammatory markers as determined by C-reactive protein and erythrocyte sedimentation rates.[IV] This inflammatory response combined with the increase in neutrofil granulocytes[IV] could potentially lead to plaque destabilization and progression of atherosclerosis. An experiment with high cholesterol-fed pigs showed no effect of G-CSF on the atherosclerotic lesions or on neutrophil infiltration in the lesions. (221) In contrast, a preclinical study in apolipoprotein E-deficient mice, demonstrated an exacerbation of the atherosclerotic lesions after treatment with G-CSF for 8 weeks. (268) This effect was only evident in mice maintained on high fat diet.

There has been some evidence that intramyocardial transplantation of skeletal myoblasts induce ventricular tachyarrhythmia in patients. (269,270) None of the clinical trials with G-CSF treatment to ischemic heart disease has indicated a pro-arrhythmic effect and an experiment in mice has even indicated a reduced inducibility of ventricular arrhythmias after G-CSF treatment when compared with controls. (271)

In conclusion, the treatment with G-CSF following STEMI and PCI seems to be safe. Still, it cannot be totally excluded that G-CSF may have contributed to the serious adverse events reported from the trials leading to an offset of the positive effects observed in animal studies.

WHY DOES G-CSF NOT IMPROVE MYOCARDIAL FUNCTION FOLLOWING STEMI?

It is remarkable that early phase clinical trials and in particular the randomized FIRSTLINE-AMI trial including 50 patients suggested a positive effect of G-CSF, whereas all randomized and double-blinded trials using G-CSF for STEMI were neutral in effect. The major differences between the FIRSTLINE-AMI and the later double blinded trials are the lack of placebo treatment in the FIRSTLINE-AMI and thus lack of blinding; and the time of G-CSF administration.

Time to G-CSF

G-CSF was administered as early as 89 min (SD 35 min) after reperfusion in the FIRSTLINE-AMI study, which is somewhat earlier than the remaining trials (Figure 9). This could explain some of the differences since experimental evidence in mice suggest a time-sensitive, direct, cardioprotective effect of G-CSF rather than a cell-mediated effect. The study indicated that the anti-apoptotic effect was significantly reduced if treatment was delayed to only 3 days post-myocardial infarction. (76) It is however important to notice that the G-CSF dose used in the mouse study was 10 to 20 times higher than the dosages used in human trials (up to 100 µg/kg).

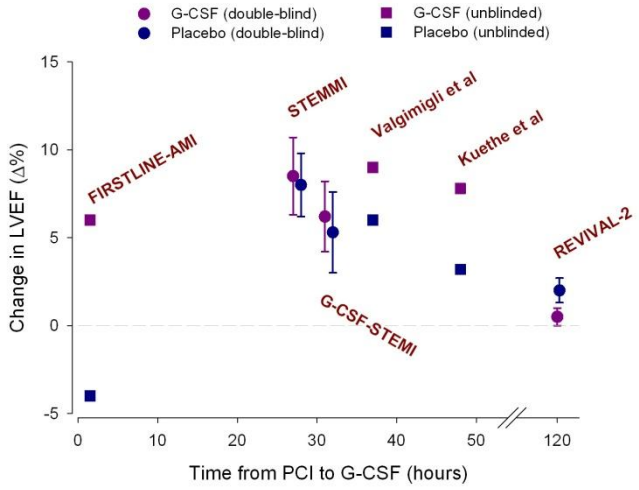

Figure 9
Recovery of ejection fraction in human trials of G-CSF after STEMI in relation to time to G-CSF. *(Adapted from Ripa et al Exp Hematol 2008;36:684 with permission from Elsevier)*

The REPAIR-AMI trial revealed a significant interaction between the absolute changes in left ventricular ejection fraction at 4 months and the time from reperfusion therapy to direct intracoronary infusion of bone marrow cell solution or placebo medium, in fact beneficial effect was confined to patients treated

later than 4 days after reperfusion. (50) This corresponds well with results from the STEMMI trial where the peak concentration of $CD34^+$ and $CD45^-/CD34^-$ mononuclear cells, was measured in peripheral blood 4 to 7 days after the initiation of G-CSF treatment.[V] Some evidence could even suggest a very late time-point of cell therapy as optimal since homing factors involved in myocardial engraftment of mobilized or infused cells (SDF-1) and vascular growth factors (VEGF-A and FGF) only increase slowly during the first weeks after acute myocardial infarction and reach maximum concentrations after 3 weeks. (32) We have performed a post hoc analysis of the STEMMI trial to address the issue regarding time to treatment in relation to outcome. (272) There were no indications in this study that the timing of G-CSF treatment in STEMI patients plays a role in the recovery of left ventricular ejection fraction (Figure 10). This result is comparable to a post-hoc analysis of the G-CSF–STEMI trial (273) concluding that G-CSF after myocardial infarction does not improve myocardial function if the cytokine is given early. The G-CSF–STEMI trial however, did not include STEMI patients treated with acute primary PCI, but patients with subacute STEMI and late revascularization (mean 32 hours after symptom onset), making a direct comparison to our data difficult.

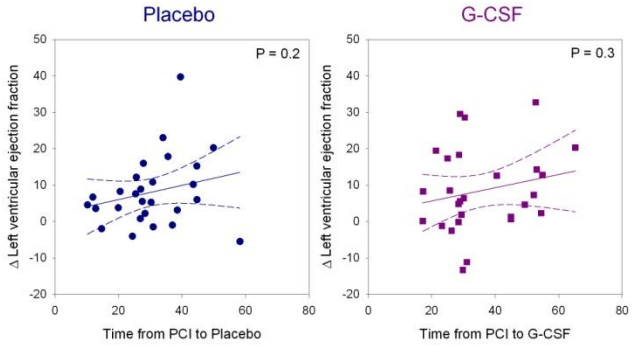

Figure 10
Association between time to treatment and recovery of left ventricular ejection fraction in the STEMMI trial. *(Reprinted from ref. 272 with permision from Elsevier)*

The G-CSF dose

The optimal dose of G-CSF remains unknown. Most clinical trials so far have pragmatically used 10 µg G-CSF/kg per day known from clinical hematology. Only a single clinical trial of patients with myocardial ischemia has addressed this issue. Ellis et al (257) randomized 18 patients with STEMI into double blind treatment with placebo (N=6), G-CSF 5 µg/kg per day (N=6), or G-CSF 10 µg/kg per day (N=6). G-CSF treatment led to a 5- to 7-fold increase in $CD34^+$ and $CD117^+$ cells with no apparent difference in mobilization between the 2 doses of G-CSF. (257) In contrast, we have found evidence of a dose-dependent cell mobilization in patients with stable ischemic heart disease. (219) One trial found dose-dependent improvement in regional myocardial function after intracoronary infusion of bone marrow mononuclear cells. (274) The direct cardioprotective effect of G-CSF seen in mice (76) could requires an even higher dose of G-CSF since this study used up to 100 µg/kg per day.

Differential mobilization of cell types

Another aspect of G-CSF treatment versus direct intracoronary cell infusion is the type of cells used. It remains puzzling that in vivo mobilization of bone marrow–derived cells by G-CSF to the circulation does not result in myocardial recovery comparable to that apparently achieved by ex vivo purification and subsequent intracoronary infusion of bone marrow–derived cells in the REPAIR-AMI trial. (50) Animal experiments indicate that MSC may be good candidates for cardiac repair (163,164,275), whereas the hematopoietic progenitor cells are less likely to improve cardiac function. (6) One hypothesis could thus be that G-CSF does not mobilize effective cell types (such as MSC) whereas bone marrow aspiration and purification yields these cells. We analyzed peripheral blood cells from the STEMMI trial to investigate this hypothesis.[V] G-CSF is known to mobilize endothelial and hemotopoietic cells from the bone marrow to the peripheral blood (page 3). (220,247) The mobilization of MSC by G-CSF is more debated. In a much cited paper Pitchford et al. showed that treatment with G-CSF did not increase PBMSC (276) and others have found similar results. (172,277) However, several other trials have found indications that G-CSF do increase the number of PBMCS. (173,278-282) Indirect evidence of bone marrow mobilization and myocardial engraftment of MSC comes from studies of mice receiving bone marrow transplantation with MSC expressing enhanced green fluorescent protein. Following acute myocardial infarction and G-CSF treatment, cells expressing enhanced green fluorescent protein and actinin were identified in the myocardium. (278) More direct evidence come from trials identifying PBMSC (typically CFU-F) in both healthy donors (humans or animals) (173,279,280) or following myocardial ischemia (281,282). Myocardial ischemia without any mobilizing treatment also seem to increase the number of PBMSC. (282) The mechanism of G-CSF induced increase in PBMSC is unknown but the observed differences in the temporal concentrations of MSC, EPC and hematopoietic stem cells in the blood following G-CSF treatment of normal mice could indicate diverse mechanisms. (279) We assessed the number of PBMSC by identification of circulating mononuclear cells negative for both the endothelial marker CD34 and the pan-leukocyte marker CD45 for reasons discussed on page 12. This identification procedure has low specificity for MSC, and to designate the whole population of circulating $CD45^-/CD34^-$ cells as putative MSC in the paper is retrospectively and according to the minimal criteria by The International Society for Cellular Therapy (141) inexact.

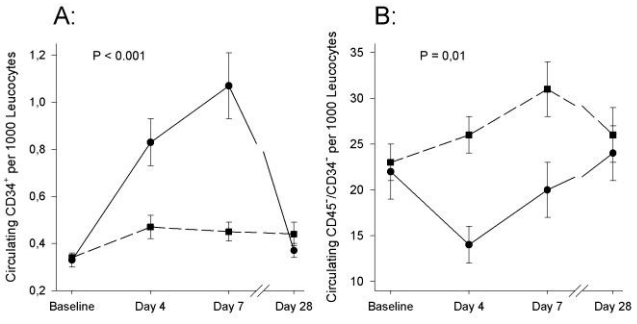

Figure 11
Ratio of (A) $CD34^+$ cells/1000 leucocytes, and (B) $CD45^-/CD34^-$ cells/1000 leucocytes in the blood during 30 days after myocardial infarction. Full line is G-CSF treatment and bracket line is placebo treatment. *(Reprinted from ref. VI with permission from Wolters Kluwer Health)*

Figure 11 shows the number of $CD34^+$ cells (panel A) and $CD45^-/CD34^-$ cells (panel B) relative to the leukocytes following treatment with G-CSF in the STEMMI trial.[V] The fraction of $CD34^+$ cells increased during G-CSF treatment, whereas the fraction of

$CD45^-/CD34^-$ cells decreased during the treatment. Also, treatment with G-CSF compared to placebo caused a shift in the subtypes of $CD45^-/CD34^-$ in the peripheral blood with a minor fraction of cells expressing CD73 (a marker expressed by MSC) and CD31 (an endothelial marker), and a larger fraction expressing the VEGF-R (an endothelial marker) and CD133 (a hematopoitic marker).[V] However, expression of several of the marker known to be expressed by both MSC and endothelial cells are not affected by G-CSF (CD105, CD166, CXCR4, CD144). This heterogenous change in subtypes of $CD45^-/CD34^-$ is difficult to interpret and is probably caused by the low sensitivity and specificity of the included markers.

It can be hypothesized, that the identified differential G-CSF mobilization of circulating $CD34^+$ and $CD45^-/CD34^-$ cells might in part explain the observed difference in therapeutic effect of G-CSF vs. intracoronary infusion of bone marrow cells (in the STEMMI vs. in the REPAIR-AMI trial). However, the results should be interpreted with caution due to the low number of patients, the exploratory nature of the design, and the low sensitivity and specificity of the surface markers for identifying discrete cell populations.

Homing of mobilized cells to the myocardium

Homing of the circulating cells into the ischemic myocardium is a prerequisite for both a paracrine mechanism and a direct incorporation and differentiation of progenitor/stem cells. Patients with ischemic heart disease seem to have impaired homing capacity and additionally G-CSF seems to impair the migratory capacity of the mobilized cells (page 13). We have roughly estimated the number of cells supplied to the ischemic myocardium (and thus available for homing) during the first week after G-CSF/placebo treatment. The purpose of this estimate was to compare our mobilizing approach with the number of cells infused in studies using an intracoronary infusion of bone marrow-derive mononuclear cells, and also to compare the number of cells mobilized in the STEMMI and the FIRSTLINE-AMI trials. For the estimate, we used cell concentrations measured at day 1, day 4, and day 7. The blood flow to ischemic myocardium was approximated at 80 ml/min in all patients based on the method used by Ince et al in the FIRSTLINE-AMI trial. (256,283) Previously, one trial found a mean flow rate of approximately 60 ml/min through the stented segment following primary PCI (284) and another trial found a mean flow of 140/118/144 ml/min in the proximal LAD/LCx/RCA and 55/51/64 ml/min in the distal segments of the same arteries. (285) More recently, Erbs et al measured a basal coronary blood flow just below 80 ml/min in the stented infarct related artery 4 days after STEMI in the REPAIR-AMI trial. (58) The estimate has the obvious limitations that differences in volume of ischemic tissue, vascular dilatation, and presence of microvascular obstruction will cause inter-patient differences in the true blood volume supplied to the ischemic area. Compared to FIRSTLINE-AMI we found almost identical numbers of $CD34^+$ cells ($2.5x10^{10}$ vs $2.8x10^{10}$). Thus, our G-CSF approach seemed to expose the myocardium to more than 100 times the number of $CD34^+$ cells infused in the REPAIR-AMI trial (50). We found no association between the estimated total number of $CD34^+$ cells supplied to the postischemic myocardium after myocardial infarction and the subsequent change in left ventricular ejection fraction.[V] An inverse association was found between the estimated number of $CD45^-/CD34^-$ cells supplied to the postischemic myocardium and the change in left ventricular ejection fraction (95% CI of regression coefficient -11.4 to -2.2, P=0.004).[V] We found similar results when using the day 7 concentration of $CD34^+$ or $CD45^-/CD34^-$ cells rather that the estimated total number of cells to predict recovery of ejection fraction (Figure 12). The association was not reproduced when using systolic wall thickening as dependent variable. This could indicate a statistical type I error.

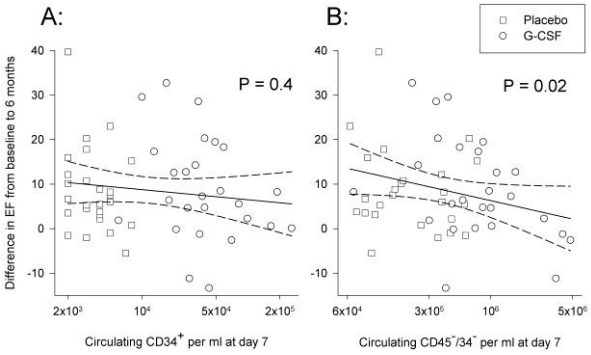

Figure 12
Association between changes in left ventricular ejection fraction during 6 months and concentration of (A) $CD34^+$, and (B) $CD45^-/CD34^-$ cells on day 7 after treatment with G-CSF or placebo. Regression line with 95% confidence interval. *Abscissa in logarithmic scale.

A causality of the association cannot be determined in an observational study, but the results may suggest that a low concentration of the $CD45^-/CD34^-$ cells in the blood is due to engraftment of the cells into the myocardium. Thus, patients with a high inert potential for myocardial homing after STEMI will have the highest degree of systolic recovery due to the engrafted cells. Of note, we found a similar inverse association between ejection fraction and $CD45^-/CD34^-$ cells in patients with chronic myocardial ischemia.[II]

It could alternatively be speculated that $CD45^-/CD34^-$ cells are not homing, but potentially reduce the recovery of the myocardial function explaining the inverse association between circulating $CD45^-/CD34^-$ cells and changes in global ventricular function.[V] A study in dogs has indicated that intra-coronary infusion of MSC could cause micro-infarctions, probably due to microvascular obstruction by the cells. (286) However, there was no biochemical or electrocardiographic evidence of myocardial ischemia during the G-CSF treatment in the STEMMI trial. In addition, circulating mononuclear cells collected after G-CSF treatment and then injected into the infarct related coronary artery in patients with STEMI did not result in any signs of myocardial damage. (287)

CONCLUSION

There is no convincing evidence that monotheraphy with G-CSF early after STEMI improves recovery of left ventricular function, despite the discrepancy in results when compared with previous animal and uncontrolled or unblinded clinical G-CSF trials. We still cannot exclude the possibility that the trials have been underpowered to detect a small difference but the recent meta-analysis makes this unlikely. (261) As for patients with chronic ischemia, it must be speculated if arteriogenesis rather that angiogenesis should be the goal of the therapy. The complex interaction between stem cell mobilization/engraftment and cytokines remains poorly understood, and the results do not exclude the possibility that G-CSF could be part of a treatment strategy combing several cytokines and/or local stem cell delivery in future trials. (288) The MAGIC Cell-5-Combicytokine Trial (*clinicaltrial.org*, NCT00501917) that was initiated March 2007 to evaluate the efficacy of combi-

nation therapy with erythropoietin and intracoronary infusion of G-CSF mobilized peripheral blood stem cells. The SITAGRAMI-Trial initiated in March 2008 aim to test a dual strategy of G-CSF in combination with an inhibition of SDF-1 degradation. (289)

5. MYOCARDIAL RECOVERY AFTER STEMI

Cardiac MRI is an attractive method for efficacy assessment in early phase clinical trials since the high accuracy and precision allows for inclusion of a minimum of patients (page 7). However, in using MRI-derived endpoints it is important to acknowledge (especially in early trials without randomized control groups) the limited knowledge of the natural course of myocardial recovery following a reperfused acute myocardial infarction with modern guideline treatment. One example is the use of G-CSF for acute myocardial infarction where early non-controlled trials postulated an effect. However, the later randomized trials showed a similar improvement in the placebo treated groups. We therefore performed a study aiming at the investigation of the short-term and long-term effects of current guideline treatment of STEMI, including successful primary PCI, in terms of left ventricular function, morphology, edema, and perfusion using cardiac MRI.[VI] Overall, we observed a substantial recovery of all investigated variables primarily within the first month after the reperfusion. Left ventricular ejection fraction increased with more that 8 percentage points (Figure 13), the systolic wall thickening in the infarct area almost doubled (Figure 14), and the perfusion of the infarcted myocardium increased with approximately 50%.[VI] These results are potentially biased by the low number of patients with a potential selection bias (n=54) and the post hoc design, but the variance of the means were still acceptable (e.g. 95% CI of the mean ejection fraction change from 4 to 9 percentage points). Furthermore, these patients were all included in a trial[IV] and we cannot exclude the possibility that the post AMI care were more careful than daily clinical practice.

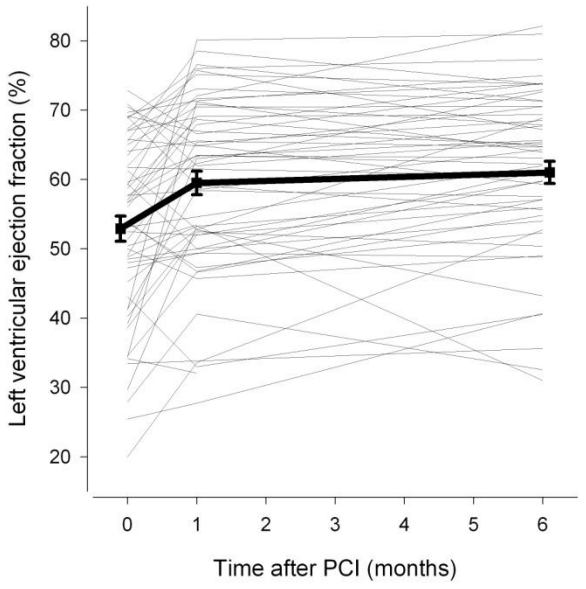

Figure 13
Change in ejection fraction. Bold line indicates mean±SE. *(Reprinted from ref. VI with permission from Elsevier)*

The results found are comparable to those of a smaller MRI study (N=22) by Baks et al (290) who found an increase in ejection fraction of 7 percentage points. Trials assessing the change in ejection fraction from angiography show minor but still substantial increases (3-6 percentage points) (291-293)

In contrast, one trial including 51 patients with myocardial infarction found no change in ejection fraction. (294) Study design and population can potentially explain some of the differences; first and perhaps most important, the baseline MRI was not performed until 5 days after the infarction, second, only 16 of the patients were treated with angioplasty, 21 with trombolysis and 14 with aspirin alone owing to late admission or diagnosis, and third, the left ventricular volumes were assessed from two long axis cine loops using the modified biplane Simpson's method, (294) whereas we assessed the volumes from short-axis cine loops (usually 10) covering the entire left ventricle.[VI] The improvement in wall thickening confirmed a previous trial of 17 patients showing a increase from 22 to 38% in the infarcted area. (295)

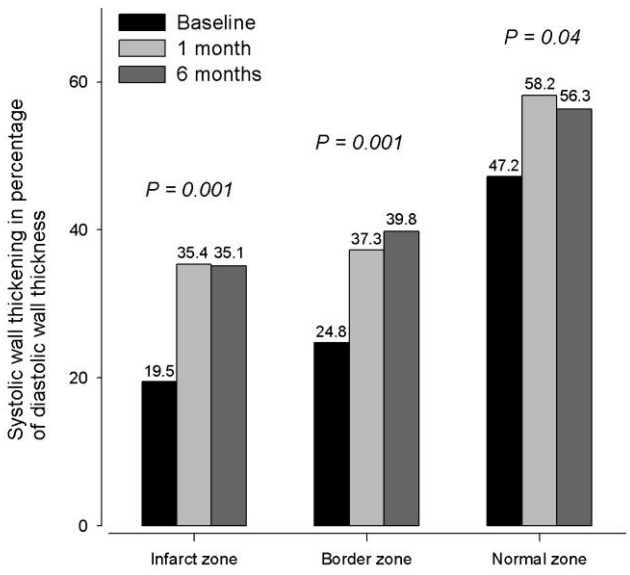

Figure 14
Mean change in systolic wall thickening efter STEMI in 3 myocardial areas.[VI]

A limitation of our study design is the 30-day time span between the initial and first follow-up examination, which does not allow firm conclusions about the precise timing of the changes observed. However, results from others suggest that the recovery of left ventricular ejection fraction primarily happens within the first week following reperfusion, (258,296,297) since these trials with a later baseline MRI observe less recovery of ejection fraction. This spontaneous recovery is probably primarily due to early myocardial stunning following the ischemic event. (298)

The infarct mass was reduced by almost 30% from baseline to 6 months follow-up,[VI] a result very similar to the results of others. (260,299,300) This is consistent with animal data showing that healing of a myocardial infarct is an ongoing process: (301) After four days central necrosis, hemorrhage and inflammation can be observed. This is followed by infarct resorption, scar formation by tissue composed of fibroblasts in a dense collagen matrix and wall thinning after six weeks. (301) There has been some suggestions that infarct size assessed by MRI in the first days after the infarc-

tion overestimates the true infarct size (302-304) perhaps due to myocardial edema in the adjacent myocardium. However, two methodologically strong studies in dogs by the group of Kim and Judd (305,306) showed a very close correlation between in vivo and ex vivo infarct size measured with MRI and infarcted regions defined by triphenyltetrazolium chloride staining from 4 hours to 8 weeks after coronary artery occlusion (both with and without reperfusion).

The results of our MRI study[VI] underscores the importance of a proper control group in trials including patients after acute myocardial infarction due to the substantial change in all measured parameters during the first month, or at the very least a good knowledge of the natural course of the disease. In addition, it appears of crucial importance that baseline examinations are performed within a narrow time window after the STEMI when comparing several populations.

6. FROM BED TO BENCH

To date most clinical trials of cells therapy have had a pragmatic design with intracoronary infusion of autologeous bone marrow-derived mononuclear cells. (307) Bone marrow-derived mononuclear cells are isolated using density gradient centrifugation following bone marrow aspiration. The bone marrow-derived mononuclear cell suspension primarily comprises nonprogenitor cells and only about 3% hematopoietic stem/progenitor cells or EPC. (42) Cells positive for the hematopoietic surface marker CD34 can also be obtained from the peripheral blood, but an experimental rat study has suggested that bone marrow-derived mononuclear cells may provide an advantage when compared to peripheral blood-derived mononuclear cells. (215,308) The modest improvement in ejection fraction seen in most clinical trials (3% in a meta-analysis (307)) has shifted the focus towards the use of more specific stem or progenitor cell lines to improve outcomes. One potentially useful cell line is the MSC, which can be isolated from the bone marrow and expanded in culture. (309-311) Allogeneic MSC has been infused intravenous in a clinical trial in patients after myocardial infarction. (312) It was primarily a safety trial, but the results indicated a positive impact of MSC on ejection fraction by MRI and by echocardiography in anterior wall infarction only. A number of other secondary endpoints (wall thickness, wall motion score index, and 6-min walk test) were unaffected by MSC.

The optimal route of delivery of cells to the heart and the potential mechanism by which cell-based therapy works also remains to be determined. Imaging based cell-tracking can potentially elucidate important mechanistic issues by determining homing, engraftment and growth of cells following transplantation. Furthermore identification of redistribution to other organs where the transplanted cells might lead to side effects is of vital importance.

The ideal imaging technique should allow for serial tracking in humans for a prolonged period of time with high spatial resolution and with the capability of tracking a few cells without affecting the cells or the organ. It is important that the marker remains in the viable cell but is quickly cleared from the tissue upon cell death.

Several in vivo imaging techniques are available and currently direct labeling with radionuclides for gamma camera imaging or PET (310,311,313) or labeling with iron particles for MRI (314) appear suitable.

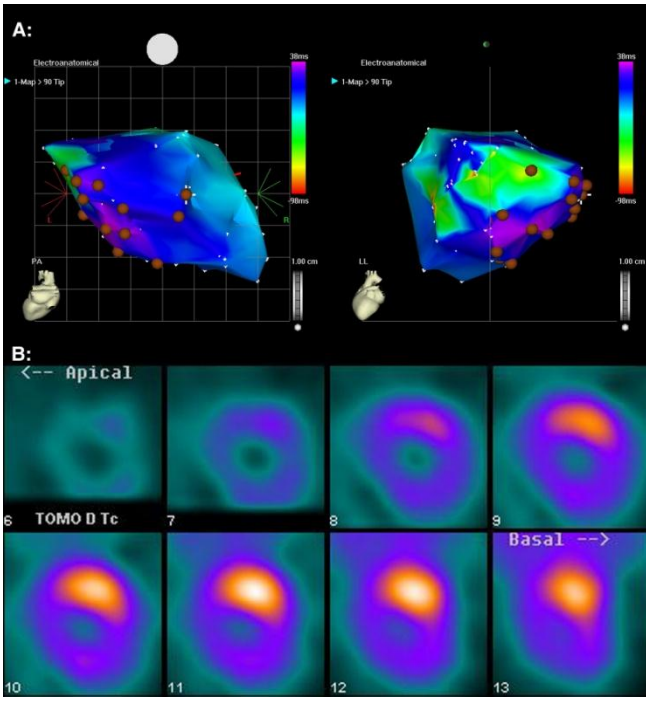

Figure 15
A, Endocardial NOGA mapping of a pig heart. The dots indicate the injection sites. B, Dual isotope SPECT images of the left ventricle in the short axis showing a hot spot of 111In activity in the anterior wall, corresponding to the injection sites in the NOGA map. *(Reprinted from ref. VII with kind permission from Springer Science+Business Media)*

Imaging of leukocyte distribution using ^{111}Indium (^{111}In) is a safe clinical routine procedure. (315) In-111 is commercially available with a half-life of 2.8 days making in vivo tracking up to 2 weeks possible. Experiments by Jin et al (316) and Gholamrezanezhad et al (317) might suggest that the radioactivity from ^{111}In could be toxic to stromal cells, however, we found no indication of radiotoxic effects on viability and/or function after labeling of human MSC with ^{111}In-tropolone. (318) Perhaps differences in culture or labeling procedures could explain these differences in results.

Previously, several studies have used indium labeling of both MSC and EPC and subsequent in vivo tracking of the radioactivity as a surrogate measure of cell engraftment and migration. These trials have been conducted assuming that radioactivity assesses living and active cells. (319-322) In one trial ^{111}In labeled progenitor cells were infused into the coronary artery in patients after acute myocardial infarction (N=20). (319) One hour after infusion of progenitor cells, a mean of 6.9±4.7% (range, 1% to 19%; n=17) of total radioactivity was detected in the heart. Radioactivity remained in the heart after 3 to 4 days, indicating homing of progenitor cells to the myocardium. (319) Several of the experimental trials have confirmed the presence of labeled cells by histology following euthanasia of the animals. (323-326)

We designed a pilot trial to investigate whether the biodistribution and retention of ex-vivo cultured MSC can be determined after direct percutaneous intramyocardial transplantation in a large animal model by ^{111}In-tropolone radiolabeling of human MSC.[VII] Labeled MSC were first transplanted into four pigs by trans-endocardial percutaneous injections. The ^{111}In activity in the heart was 35% (±11%) of the total activity in the pig one hour after injection of viable ^{111}In labeled MSC,[VII] compared to only 6.9±4.7% after intracoronary infusion (319) and from 11.3±3%

(327) to 20.7±2.3% (324) after trans-epicardiel injection. SPECT imaging identified the ^{111}In within the myocardium corresponding to the locations of the intramyocardial injections (Figure 15). Whole body scintigraphy revealed focal indium accumulations in the cardiac region up to 6 days after injection.[VII] Myocardium with high radioactivity was analyzed by fluorescence in situ hybridization (FISH) and microscopy after euthanization of the animals. No human MSCs were identified with FISH, and microscopy identified widespread necrosis and acute inflammation. Two new pigs were then treated with immunosuppressive therapy to diminish the host-versus-graft reaction observed in the first 4 pigs, but injection of MSC still lead to a similar pattern with focal indium accumulation, and inflammation but no human MSCs could be identified. Two additional pigs were injected with ^{111}In-tropolone (without cells) to test the tissue response to the gamma radiation from ^{111}In. In these two pigs radioactive tissue samples, identified using a gamma detection probe, showed normal myocardium without inflammation.[VII] This indicates that neither the gamma radiation nor the intramyocardial injection causes the inflammation and tissue-damage, which is in concordance with our previous experience from intramyocardial injections. (218) A last pig was injected with dead ^{111}In labeled cells. The clearance of radioactivity of injected dead cells and of ^{111}In alone appeared faster initially compared to that of viable cells, but retention after injection of viable cells, dead cells and ^{111}In followed a very similar pattern (Figure 16).

The results of this trial were potentially biased by several factors. We only included few animals in a prospective design making this a hypothesis generating trial. However, we did consistently in 6 animals observe intense radioactivity despite disappearance of the cells. In our opinion, a very high specificity (close to 100%) should be demanded of this labeling method, and our results are in conflict with this. Another limitation is the FISH method; we have not quantitatively determined the sensitivity of the method in our setup and thus cannot exclude the possibility that a few of the cells were present despite the negative FISH result. However, based on the radioactivity in the tissue-sample excised for FISH analysis, we would expect >10^5 human cells per gram tissue (assessed using our initial mean activity of 1.4 Bq/cell).

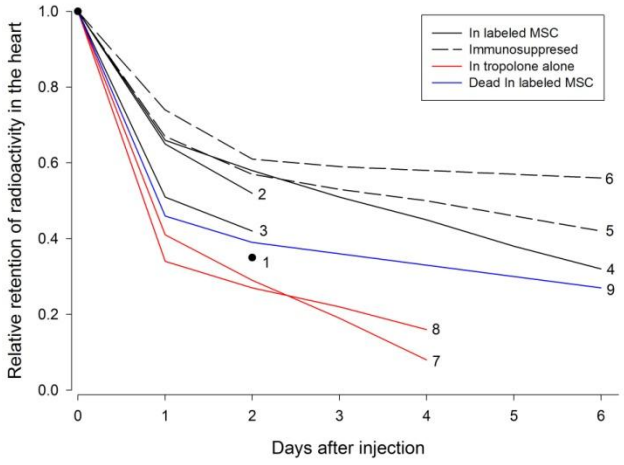

Figure 16
Relative retention of radioactivity after correction for decay. *(Reprinted from ref. VII with kind permission from Springer Science+Business Media)*

In conclusion, the pilot study generates two important hypotheses.[VII] First, as radioactivity from ^{111}In-labeled cells stays in the myocardium for a long time despite the disappearance of transplanted cells, clinical use of ^{111}In-labeled cells for monitoring of MSC in the human heart seems problematic unless viability can be determined by another method. Second, xenografting of human MSC into a pig leads to an inflammatory response and fast degradation of the cells even under pharmacologic immunosuppression.[VII]

Our results indicate that an alternative imaging modality is warranted. Iron-oxide labeling for MRI tracking of injected cells has appeared as a suitable alternative to indium labeling, with the possibility of even longer follow-up. However, recent results very similar to ours using iron labeling were reported. (328,329) After 3-4 weeks no transplanted cells were detected. Instead a continued enhanced magnetic resonance signal was found from cardiac macrophages that engulfed the labeling particles suggesting that iron-oxide labeling is also an unreliable marker for monitoring cell survival and migration. (328,329)

Reporter gene imaging using clinical PET is another promising modality. (330) The reporter gene is expected to be lost after cell death providing a more specific signal, but the technology still needs further work.

7. GENERAL CONCLUSIONS AND FUTURE DIRECTIONS

The objective of our investigations was to evaluate G-CSF as a cell-based therapy for ischemic heart disase.

We found no effects of combined treatment with G-CSF and VEGF-A-gene therapy in patients with chronic ischemic heart disease in a small scale clinical trial. Concordantly, we must conclude that if G-CSF and VEGF-A have an effect on these patients, the effect is most likely very small. We could not identify any inherent factors in the blood or genetic variations in the VEGF-A gene that could select optimal patients for cell-based therapies.

When randomizing patients with STEMI to G-CSF or placebo we found no clinical effect of G-CSF, but we did observe a substantial recovery of myocardial function following current guideline-based therapy of STEMI. This ´natural´ effect could potentially explain the apperant effect of G-CSF previously reported in non-controlled trials. Thus, our results did not support our pretrial hypothesis that cell mobilization alone or in combination with modulating molecules would result in clinical effective cardiac regeneration.

Several clinical trials of G-CSF for ischemic heart disease were published and planned prior to the presentation of the STEMMI and the RIVIVAL-2 results. The group behind the FIRSTLINE-AMI trial even planned a large-scale multicenter clinical trial of G-CSF after acute myocardial infarction based on their positive results in a non-blinded trial. Our trials brought science past G-CSF as monotherapy for ischemic heart disease despite the negative results.

The heart is a complex organ composed of muscle and non-muscle cells integrated into a three-dimensional structure. Cardiac regeneration will probably require more than simply supplying the right cell to the right tissue, at the right time. So far, clinical trials have had a pragmatic design using the cell types that are readily available. This probably leads to extensive cell death and inadequate integration in a hostile immunoreactive, ischemic or necrotic environment explaining the neutral or small effects observed in clinical trials.

Defining the factors present in the hostile microenvironment of injured myocardium that limit the homing, functional engraftment and survival of transplanted cells will be essential for guid-

ing the development of stem-cell-based therapies. Unfortunately, no good method for long-term in vivo imaging of transplanted cells exists. To complicate matters even more, the results in clinical trials are potentially biased by a significant change in both morphology, function, and perfusion following PCI treated acute myocardial infarction without cell therapy.

Tissue engineering (331,332) combining cells with artificial or natural scaffolds, or intramyocardial injection of combinations of cell types and/or cytokines may be more effective than a single intracoronary injection of single-cell suspensions. Alternatively, long-time engraftment and survival of transplanted cells may not be necessary if paracrine effects are the main mechanism of cell-based therapies. In that case, identification and administration of the secreted active components could be more appropriate than cell transplantation. As the many remaining questions regarding cardiac regeneration are elucidated, meticulously designed clinical trials should proceed with caution and with a paramount concern for patient safety. Ultimately larger trials are needed to answer the key question if improvement in surrogate endpoints translates into improvement in clinical endpoints such as mortality and morbidity. The publication of both positive and negative trial results provides the research community the important opportunity to progress.

Hopefully, the next decade will make the intuitively attractive concept of regenerating the broken heart a reality.

8. SUMMARY

Cell based therapy for ischemic heart disease has the potential to reduce post infarct heart failure and chronic ischemia.

Treatment with granulocyte-colony stimulating factor (G-CSF) mobilizes cells from the bone marrow to the peripheral blood. Some of these cells are putative stem or progenitor cells. G-CSF is injected subcutaneously. This therapy is intuitively attractive compared to other cell based techniques since repeated catheterizations and ex vivo cell purification and expansion are avoided. Previous preclinical and early clinical trials have indicated that treatment with G-CSF leads to improved myocardial perfusion and function in acute or chronic ischemic heart disease.

The hypothesis of this thesis is that patient with ischemic heart disease will benefit from G-CSF therapy. We examined this hypothesis in two clinical trials with G-CSF treatment to patients with either acute myocardial infarction or severe chronic ischemic heart disease. In addition, we assed a number of factors that could potentially affect the effect of cell based therapy. Finally, we intended to develop a method for in vivo cell tracking in the heart.

Our research showed that subcutaneous G-CSF along with gene therapy do not improve myocardial function in patients with chronic ischemia despite a large increase in circulation bone marrow-derived cells. Also, neither angina pectoris nor exercise capacity was improved compared to placebo treatment. We could not identify differences in angiogenic factors or bone marrow-derived cells in the blood that could explain the neutral effect of G-CSF.

Next, we examined G-CSF as adjunctive therapy following ST segment elevation myocardial infarction. We did not find any effect of G-CSF neither on the primary endpoint - regional myocardial function - nor on left ventricular ejection fraction (secondary endpoint) compared to placebo treatment. In subsequent analyses, we found significant differences in the types of cells mobilized from the bone marrow by G-CSF. This could explain why intracoronary injections of unfractionated bone marrow-derived cells have more effect that mobilization with G-CSF.

A number of other factors could explain the neutral effect of G-CSF in our trial compared to previous studies. These factors include timing of the treatment, G-CSF dose, and study population. It is however, remarkable that the changes in our G-CSF group are comparable to the results of previous non-blinded studies, whereas the major differences are in the control/placebo groups. We found that ejection fraction, wall motion, edema, perfusion, and infarct size all improve significantly in the first month following ST-segment myocardial infarction with standard guideline treatment (including acute mechanical re¬vascularization), but without cell therapy. This is an important factor to take into account when assessing the results of non-controlled trials.

Finally, we found that ex vivo labeling of cells with indium-111 for in vivo cell tracking after intramyocardial injection is problematic. In our hand, a significant amount of indium-111 remained in the myocardium despite cell death. It is difficult to determine viability of the cells after injection in human trials, and it is thus complicated to determine if the activity in the myocardium tracks viable cells.

Cell based therapy is still in the explorative phase, but based on the intense research within this field it is our hope that the clinical relevance of the therapy can be determined in the foreseeable future. Ultimately, this will require large randomized, double-blind and placebo-controlled trials with “hard” clinical endpoints like mortality and morbidity.

9. REFERENCES

1. McCulloch EA, Till JE. The radiation sensitivity of normal mouse bone marrow cells, determined by quantitative marrow transplantation into irradiated mice. Radiat Res 1960;13:115-25.
2. Asahara T, Masuda H, Takahashi T, Kalka C, Pastore C, Silver M, Kearne M, Magner M, Isner JM. Bone marrow origin of endothelial progenitor cells responsible for postnatal vasculogenesis in physiological and pathological neovascularization. Circ Res 1999;85:221-8.
3. Asahara T, Murohara T, Sullivan A, Silver M, van der ZR, Li T, Witzenbichler B, Schatteman G, Isner JM. Isolation of putative progenitor endothelial cells for angiogenesis. Science 1997;275:964-7.
4. Orlic D, Kajstura J, Chimenti S, Jakoniuk I, Anderson SM, Li B, Pickel J, McKay R, Nadal-Ginard B, Bodine DM, Leri A, Anversa P. Bone marrow cells regenerate infarcted myocardium. Nature 2001;410:701-5.
5. Nygren JM, Jovinge S, Breitbach M, Sawen P, Roll W, Hescheler J, Taneera J, Fleischmann BK, Jacobsen SE. Bone marrow-derived hematopoietic cells generate cardiomyocytes at a low frequency through cell fusion, but not transdifferentiation. Nat Med 2004;10:494-501.
6. Murry CE, Soonpaa MH, Reinecke H, Nakajima H, Nakajima HO, Rubart M, Pasumarthi KB, Virag JI, Bartelmez SH, Poppa V, Bradford G, Dowell JD, Williams DA, Field LJ. Haematopoietic stem cells do not transdifferentiate into cardiac myocytes in myocardial infarcts. Nature 2004;428:664-8.
7. Kastrup J, Jørgensen E, Drvota V. Vascular growth factor and gene therapy to induce new vessels in the ischemic myocardium. Therapeutic angiogenesis. Scand Cardiovasc J 2001;35:291-6.

8. Hendel RC, Henry TD, Rocha-Singh K, Isner JM, Kereiakes DJ, Giordano FJ, Simons M, Bonow RO. Effect of intracoronary recombinant human vascular endothelial growth factor on myocardial perfusion: evidence for a dose-dependent effect. Circulation 2000;101:118-21.
9. Henry TD, Annex BH, McKendall GR, Azrin MA, Lopez JJ, Giordano FJ, Shah PK, Willerson JT, Benza RL, Berman DS, Gibson CM, Bajamonde A, Rundle AC, Fine J, McCluskey ER. The VIVA trial: Vascular endothelial growth factor in Ischemia for Vascular Angiogenesis. Circulation 2003;107:1359-65.
10. Henry TD, Rocha-Singh K, Isner JM, Kereiakes DJ, Giordano FJ, Simons M, Losordo DW, Hendel RC, Bonow RO, Eppler SM, Zioncheck TF, Holmgren EB, McCluskey ER. Intracoronary administration of recombinant human vascular endothelial growth factor to patients with coronary artery disease. Am Heart J 2001;142:872-80.
11. Schumacher B, Pecher P, von Specht BU, Stegmann T. Induction of neoangiogenesis in ischemic myocardium by human growth factors: first clinical results of a new treatment of coronary heart disease. Circulation 1998;97:645-50.
12. Laham RJ, Chronos NA, Pike M, Leimbach ME, Udelson JE, Pearlman JD, Pettigrew RI, Whitehouse MJ, Yoshizawa C, Simons M. Intracoronary basic fibroblast growth factor (FGF-2) in patients with severe ischemic heart disease: results of a phase I open-label dose escalation study. J Am Coll Cardiol 2000;36:2132-9.
13. Laham RJ, Sellke FW, Edelman ER, Pearlman JD, Ware JA, Brown DL, Gold JP, Simons M. Local perivascular delivery of basic fibroblast growth factor in patients undergoing coronary bypass surgery: results of a phase I randomized, double-blind, placebo-controlled trial. Circulation 1999;100:1865-71.
14. Simons M, Annex BH, Laham RJ, Kleiman N, Henry T, Dauerman H, Udelson JE, Gervino EV, Pike M, Whitehouse MJ, Moon T, Chronos NA. Pharmacological treatment of coronary artery disease with recombinant fibroblast growth factor-2: double-blind, randomized, controlled clinical trial. Circulation 2002;105:788-93.
15. Sellke FW, Laham RJ, Edelman ER, Pearlman JD, Simons M. Therapeutic angiogenesis with basic fibroblast growth factor: technique and early results. Ann Thorac Surg 1998;65:1540-4.
16. Unger EF, Goncalves L, Epstein SE, Chew EY, Trapnell CB, Cannon RO, III, Quyyumi AA. Effects of a single intracoronary injection of basic fibroblast growth factor in stable angina pectoris. Am J Cardiol 2000;85:1414-9.
17. Ruel M, Laham RJ, Parker JA, Post MJ, Ware JA, Simons M, Sellke FW. Long-term effects of surgical angiogenic therapy with fibroblast growth factor 2 protein. J Thorac Cardiovasc Surg 2002;124:28-34.
18. Udelson JE, Dilsizian V, Laham RJ, Chronos N, Vansant J, Blais M, Galt JR, Pike M, Yoshizawa C, Simons M. Therapeutic angiogenesis with recombinant fibroblast growth factor-2 improves stress and rest myocardial perfusion abnormalities in patients with severe symptomatic chronic coronary artery disease. Circulation 2000;102:1605-10.
19. Yla-Herttuala S, Alitalo K. Gene transfer as a tool to induce therapeutic vascular growth. Nat Med 2003;9:694-701.
20. Symes JF, Losordo DW, Vale PR, Lathi KG, Esakof DD, Mayskiy M, Isner JM. Gene therapy with vascular endothelial growth factor for inoperable coronary artery disease. Ann Thorac Surg 1999;68:830-6.
21. Rosengart TK, Lee LY, Patel SR, Sanborn TA, Parikh M, Bergman GW, Hachamovitch R, Szulc M, Kligfield PD, Okin PM, Hahn RT, Devereux RB, Post MR, Hackett NR, Foster T, Grasso TM, Lesser ML, Isom OW, Crystal RG. Angiogenesis gene therapy: phase I assessment of direct intramyocardial administration of an adenovirus vector expressing VEGF121 cDNA to individuals with clinically significant severe coronary artery disease. Circulation 1999;100:468-74.
22. Grines CL, Watkins MW, Helmer G, Penny W, Brinker J, Marmur JD, West A, Rade JJ, Marrott P, Hammond HK, Engler RL. Angiogenic Gene Therapy (AGENT) trial in patients with stable angina pectoris. Circulation 2002;105:1291-7.
23. Losordo DW, Vale PR, Hendel RC, Milliken CE, Fortuin FD, Cummings N, Schatz RA, Asahara T, Isner JM, Kuntz RE. Phase 1/2 placebo-controlled, double-blind, dose-escalating trial of myocardial vascular endothelial growth factor 2 gene transfer by catheter delivery in patients with chronic myocardial ischemia. Circulation 2002;105:2012-8.
24. Sylvén C, Sarkar N, Ruck A, Drvota V, Hassan SY, Lind B, Nygren A, Kallner Q, Blomberg P, van der LJ, Lindblom D, Brodin LA, Islam KB. Myocardial Doppler tissue velocity improves following myocardial gene therapy with VEGF-A165 plasmid in patients with inoperable angina pectoris. Coron Artery Dis 2001;12:239-43.
25. Vale PR, Losordo DW, Milliken CE, McDonald MC, Gravelin LM, Curry CM, Esakof DD, Maysky M, Symes JF, Isner JM. Randomized, single-blind, placebo-controlled pilot study of catheter-based myocardial gene transfer for therapeutic angiogenesis using left ventricular electromechanical mapping in patients with chronic myocardial ischemia. Circulation 2001;103:2138-43.
26. Stewart DJ, Hilton JD, Arnold JM, Gregoire J, Rivard A, Archer SL, Charbonneau F, Cohen E, Curtis M, Buller CE, Mendelsohn FO, Dib N, Page P, Ducas J, Plante S, Sullivan J, Macko J, Rasmussen C, Kessler PD, Rasmussen HS. Angiogenic gene therapy in patients with nonrevascularizable ischemic heart disease: a phase 2 randomized, controlled trial of AdVEGF(121) (AdVEGF121) versus maximum medical treatment. Gene Ther 2006;13:1503-11.
27. Kastrup J, Jørgensen E, Ruck A, Tägil K, Glogar D, Ruzyllo W, Bøtker HE, Dudek D, Drvota V, Hesse B, Thuesen L, Blomberg P, Gyöngyösi M, Sylvén C. Direct intramyocardial plasmid vascular endothelial growth factor-A165 gene therapy in patients with stable severe angina pectoris A randomized double-blind placebo-controlled study: the Euroinject One trial. J Am Coll Cardiol 2005;45:982-8.
28. Kastrup J, Jørgensen E, Drvota V, Thuesen L, Bøtker HE, Gyöngyösi M, Glogar D, Rück A, Bin Islam K, Sylvén C. Intramyocardial Injection of Genes with a Novel Percutaneous Technique: Initial Safety Data of the Euroinject One Study. Heart Drug 2001;1:299-304.
29. Gyöngyösi M, Khorsand A, Zamini S, Sperker W, Strehblow C, Kastrup J, Jørgensen E, Hesse B, Tägil K, Bøtker HE, Ruzyllo W, Teresinska A, Dudek D, Hubalewska A, Ruck A, Nielsen SS, Graf S, Mundigler G, Novak J, Sochor H, Maurer G, Glogar D, Sylvén C. NOGA-guided analysis of regional myocardial perfusion abnormalities treated with intramyocardial injections of plasmid encoding vascular endothelial growth factor A-165 in patients with chronic myocardial ischemia: subanalysis of the EUROINJECT-ONE multicenter double-blind randomized study. Circulation 2005;112:I157-I165.
30. Kajstura J, Rota M, Whang B, Cascapera S, Hosoda T, Bearzi C, Nurzynska D, Kasahara H, Zias E, Bonafe M, Nadal-Ginard B, Torella D, Nascimbene A, Quaini F, Urbanek K, Leri A, Anversa P. Bone marrow cells differentiate in cardiac cell li-

neages after infarction independently of cell fusion. Circ Res 2005;96:127-37.
31. Shintani S, Murohara T, Ikeda H, Ueno T, Honma T, Katoh A, Sasaki K, Shimada T, Oike Y, Imaizumi T. Mobilization of endothelial progenitor cells in patients with acute myocardial infarction. Circulation 2001;103:2776-9.
32. Wang Y, Johnsen HE, Mortensen S, Bindslev L, Ripa RS, Haack-Sørensen M, Jørgensen E, Fang W, Kastrup J. Changes in circulating mesenchymal stem cells, stem cell homing factor, and vascular growth factors in patients with acute ST elevation myocardial infarction treated with primary percutaneous coronary intervention. Heart 2006;92:768-74.
33. Wojakowski W, Tendera M, Michalowska A, Majka M, Kucia M, Maslankiewicz K, Wyderka R, Ochala A, Ratajczak MZ. Mobilization of CD34/CXCR4+, CD34/CD117+, c-met+ stem cells, and mononuclear cells expressing early cardiac, muscle, and endothelial markers into peripheral blood in patients with acute myocardial infarction. Circulation 2004;110:3213-20.
34. Leone AM, Rutella S, Bonanno G, Contemi AM, de Ritis DG, Giannico MB, Rebuzzi AG, Leone G, Crea F. Endogenous G-CSF and CD34+ cell mobilization after acute myocardial infarction. Int J Cardiol 2006;111:202-8.
35. Werner N, Kosiol S, Schiegl T, Ahlers P, Walenta K, Link A, Bohm M, Nickenig G. Circulating endothelial progenitor cells and cardiovascular outcomes. N Engl J Med 2005;353:999-1007.
36. Kocher AA, Schuster MD, Szabolcs MJ, Takuma S, Burkhoff D, Wang J, Homma S, Edwards NM, Itescu S. 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.
37. 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.
38. Dohmann HF, Perin EC, Takiya CM, Silva GV, Silva SA, Sousa AL, Mesquita CT, Rossi MI, Pascarelli BM, Assis IM, Dutra HS, Assad JA, Castello-Branco RV, Drummond C, Dohmann HJ, Willerson JT, Borojevic R. Transendocardial autologous bone marrow mononuclear cell injection in ischemic heart failure: postmortem anatomicopathologic and immunohistochemical findings. Circulation 2005;112:521-6.
39. Strauer BE, Brehm M, Zeus T, Bartsch T, Schannwell C, Antke C, Sorg RV, Kogler G, Wernet P, Muller HW, Kostering M. 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.
40. Fuchs S, Kornowski R, Weisz G, Satler LF, Smits PC, Okubagzi P, Baffour R, Aggarwal A, Weissman NJ, Cerqueira M, Waksman R, Serrruys P, Battler A, Moses JW, Leon MB, Epstein SE. Safety and feasibility of transendocardial autologous bone marrow cell transplantation in patients with advanced heart disease. Am J Cardiol 2006;97:823-9.
41. Fuchs S, Satler LF, Kornowski R, Okubagzi P, Weisz G, Baffour R, Waksman R, Weissman NJ, Cerqueira M, Leon MB, Epstein SE. Catheter-based autologous bone marrow myocardial injection in no-option patients with advanced coronary artery disease: a feasibility study. J Am Coll Cardiol 2003;41:1721-4.
42. Perin EC, Dohmann HF, Borojevic R, Silva SA, Sousa AL, Mesquita CT, Rossi MI, Carvalho AC, Dutra HS, Dohmann HJ, Silva GV, Belem L, Vivacqua R, Rangel FO, Esporcatte R, Geng YJ, Vaughn WK, Assad JA, Mesquita ET, Willerson JT. Transendocardial, autologous bone marrow cell transplantation for severe, chronic ischemic heart failure. Circulation 2003;107:2294-302.
43. Perin EC, Dohmann HF, Borojevic R, Silva SA, Sousa AL, Silva GV, Mesquita CT, Belem L, Vaughn WK, Rangel FO, Assad JA, Carvalho AC, Branco RV, Rossi MI, Dohmann HJ, Willerson JT. Improved exercise capacity and ischemia 6 and 12 months after transendocardial injection of autologous bone marrow mononuclear cells for ischemic cardiomyopathy. Circulation 2004;110:II213-II218.
44. Strauer BE, Brehm M, Zeus T, Kostering M, Hernandez A, Sorg RV, Kogler G, Wernet P. Repair of infarcted myocardium by autologous intracoronary mononuclear bone marrow cell transplantation in humans. Circulation 2002;106:1913-8.
45. Stamm C, Westphal B, Kleine HD, Petzsch M, Kittner C, Klinge H, Schumichen C, Nienaber CA, Freund M, Steinhoff G. Autologous bone-marrow stem-cell transplantation for myocardial regeneration. Lancet 2003;361:45-6.
46. Assmus B, Schächinger V, Teupe C, Britten M, Lehmann R, Dobert N, Grunwald F, Aicher A, Urbich C, Martin H, Hoelzer D, Dimmeler S, Zeiher AM. Transplantation of Progenitor Cells and Regeneration Enhancement in Acute Myocardial Infarction (TOPCARE-AMI). Circulation 2002;106:3009-17.
47. Schächinger V, Assmus B, Britten MB, Honold J, Lehmann R, Teupe C, Abolmaali ND, Vogl TJ, Hofmann WK, Martin H, Dimmeler S, Zeiher AM. 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.
48. Wollert KC, Meyer GP, Lotz J, Ringes-Lichtenberg S, Lippolt P, Breidenbach C, Fichtner S, Korte T, Hornig B, Messinger D, Arseniev L, Hertenstein B, Ganser A, Drexler H. Intracoronary autologous bone-marrow cell transfer after myocardial infarction: the BOOST randomised controlled clinical trial. Lancet 2004;364:141-8.
49. Fernandez-Aviles F, San Roman JA, Garcia-Frade J, Fernandez ME, Penarrubia MJ, de la FL, Gomez-Bueno M, Cantalapiedra A, Fernandez J, Gutierrez O, Sanchez PL, Hernandez C, Sanz R, Garcia-Sancho J, Sanchez A. Experimental and clinical regenerative capability of human bone marrow cells after myocardial infarction. Circ Res 2004;95:742-8.
50. Schächinger V, Erbs S, Elsasser A, Haberbosch W, Hambrecht R, Holschermann H, Yu J, Corti R, Mathey DG, Hamm CW, Suselbeck T, Assmus B, Tonn T, Dimmeler S, Zeiher AM. Intracoronary bone marrow-derived progenitor cells in acute myocardial infarction. N Engl J Med 2006;355:1210-21.
51. Lunde K, Solheim S, Aakhus S, Arnesen H, Abdelnoor M, Egeland T, Endresen K, Ilebekk A, Mangschau A, Fjeld JG, Smith HJ, Taraldsrud E, Grogaard HK, Bjornerheim R, Brekke M, Muller C, Hopp E, Ragnarsson A, Brinchmann JE, Forfang K. Intracoronary injection of mononuclear bone marrow cells in acute myocardial infarction. N Engl J Med 2006;355:1199-209.
52. Janssens S, Dubois C, Bogaert J, Theunissen K, Deroose C, Desmet W, Kalantzi M, Herbots L, Sinnaeve P, Dens J, Maertens J, Rademakers F, Dymarkowski S, Gheysens O, Van Cleemput J, Bormans G, Nuyts J, Belmans A, Mortelmans L, Boogaerts M, Van de WF. 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.

53. Tendera M, Wojakowski W, Ruzyllo W, Chojnowska L, Kepka C, Tracz W, Musialek P, Piwowarska W, Nessler J, Buszman P, Grajek S, Breborowicz P, Majka M, Ratajczak MZ. Intracoronary infusion of bone marrow-derived selected CD34+CXCR4+ cells and non-selected mononuclear cells in patients with acute STEMI and reduced left ventricular ejection fraction: results of randomized, multicentre Myocardial Regeneration by Intracoronary Infusion of Selected Population of Stem Cells in Acute Myocardial Infarction (REGENT) Trial. Eur Heart J 2009;30:1313-21.
54. Hirsch A, Nijveldt R, van d, V, Tijssen JG, van der Giessen WJ, Tio RA, Waltenberger J, Ten Berg JM, Doevendans PA, Aengevaeren WR, Zwaginga JJ, Biemond BJ, van Rossum AC, Piek JJ, Zijlstra F. Intracoronary Infusion of Mononuclear Cells From Bone Marrow or Peripheral Blood Compared With Standard Therapy in Patients After Acute Myocardial Infarction Treated by Primary Percutaneous Coronary Intervention: Results of the Randomized Controlled HEBE Trial. Eur Heart J In press 2010.
55. Schächinger V, Erbs S, Elsasser A, Haberbosch W, Hambrecht R, Holschermann H, Yu J, Corti R, Mathey DG, Hamm CW, Suselbeck T, Werner N, Haase J, Neuzner J, Germing A, Mark B, Assmus B, Tonn T, Dimmeler S, Zeiher AM. Improved clinical outcome after intracoronary administration of bone-marrow-derived progenitor cells in acute myocardial infarction: final 1-year results of the REPAIR-AMI trial. Eur Heart J 2006;27:2775-83.
56. Assmus B, Rolf A, Erbs S, Elsasser A, Haberbosch W, Hambrecht R, Tillmanns H, Yu J, Corti R, Mathey DG, Hamm CW, Suselbeck T, Tonn T, Dimmeler S, Dill T, Zeiher AM, Schachinger V. Clinical outcome 2 years after intracoronary administration of bone marrow-derived progenitor cells in acute myocardial infarction. Circ Heart Fail 2010;3:89-96.
57. Meyer GP, Wollert KC, Lotz J, Steffens J, Lippolt P, Fichtner S, Hecker H, Schaefer A, Arseniev L, Hertenstein B, Ganser A, Drexler H. 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.
58. Erbs S, Linke A, Schachinger V, Assmus B, Thiele H, Diederich KW, Hoffmann C, Dimmeler S, Tonn T, Hambrecht R, Zeiher AM, Schuler G. Restoration of microvascular function in the infarct-related artery by intracoronary transplantation of bone marrow progenitor cells in patients with acute myocardial infarction: the Doppler Substudy of the Reinfusion of Enriched Progenitor Cells and Infarct Remodeling in Acute Myocardial Infarction (REPAIR-AMI) trial. Circulation 2007;116:366-74.
59. van Ramshorst J., Bax JJ, Beeres SL, bbets-Schneider P, Roes SD, Stokkel MP, de RA, Fibbe WE, Zwaginga JJ, Boersma E, Schalij MJ, Atsma DE. Intramyocardial bone marrow cell injection for chronic myocardial ischemia: a randomized controlled trial. JAMA 2009;301:1997-2004.
60. Ripa RS, Wang Y, Jørgensen E, Kastrup J. [Therapy with stem cells for acute ischemic heart disease: myocardial regeneration?]. Ugeskr Laeger 2007;169:119-22.
61. Johnsen HE. Clinical practice and future needs in recombinant human granulocyte colony-stimulating factor treatment: a review of randomized trials in clinical haemato-oncology. J Int Med Res 2001;29:87-99.
62. Avalos BR. Molecular analysis of the granulocyte colony-stimulating factor receptor. Blood 1996;88:761-77.
63. Dong F, Larner AC. Activation of Akt kinase by granulocyte colony-stimulating factor (G-CSF): evidence for the role of a tyrosine kinase activity distinct from the Janus kinases. Blood 2000;95:1656-62.
64. Anderlini P, Donato M, Chan KW, Huh YO, Gee AP, Lauppe MJ, Champlin RE, Korbling M. Allogeneic blood progenitor cell collection in normal donors after mobilization with filgrastim: the M.D. Anderson Cancer Center experience. Transfusion 1999;39:555-60.
65. Nagasawa T, Kikutani H, Kishimoto T. Molecular cloning and structure of a pre-B-cell growth-stimulating factor. Proc Natl Acad Sci U S A 1994;91:2305-9.
66. Deichmann M, Kronenwett R, Haas R. Expression of the human immunodeficiency virus type-1 coreceptors CXCR-4 (fusin, LESTR) and CKR-5 in CD34+ hematopoietic progenitor cells. Blood 1997;89:3522-8.
67. Petit I, Szyper-Kravitz M, Nagler A, Lahav M, Peled A, Habler L, Ponomaryov T, Taichman RS, renzana-Seisdedos F, Fujii N, Sandbank J, Zipori D, Lapidot T. G-CSF induces stem cell mobilization by decreasing bone marrow SDF-1 and up-regulating CXCR4. Nat Immunol 2002;3:687-94.
68. Semerad CL, Christopher MJ, Liu F, Short B, Simmons PJ, Winkler I, Levesque JP, Chappel J, Ross FP, Link DC. G-CSF potently inhibits osteoblast activity and CXCL12 mRNA expression in the bone marrow. Blood 2005;106:3020-7.
69. Kawabata K, Ujikawa M, Egawa T, Kawamoto H, Tachibana K, Iizasa H, Katsura Y, Kishimoto T, Nagasawa T. A cell-autonomous requirement for CXCR4 in long-term lymphoid and myeloid reconstitution. Proc Natl Acad Sci U S A 1999;96:5663-7.
70. Flomenberg N, DiPersio J, Calandra G. Role of CXCR4 chemokine receptor blockade using AMD3100 for mobilization of autologous hematopoietic progenitor cells. Acta Haematol 2005;114:198-205.
71. Nervi B, Link DC, DiPersio JF. Cytokines and hematopoietic stem cell mobilization. J Cell Biochem 2006;99:690-705.
72. Levesque JP, Hendy J, Takamatsu Y, Williams B, Winkler IG, Simmons PJ. Mobilization by either cyclophosphamide or granulocyte colony-stimulating factor transforms the bone marrow into a highly proteolytic environment. Exp Hematol 2002;30:440-9.
73. Levesque JP, Takamatsu Y, Nilsson SK, Haylock DN, Simmons PJ. Vascular cell adhesion molecule-1 (CD106) is cleaved by neutrophil proteases in the bone marrow following hematopoietic progenitor cell mobilization by granulocyte colony-stimulating factor. Blood 2001;98:1289-97.
74. Heissig B, Hattori K, Dias S, Friedrich M, Ferris B, Hackett NR, Crystal RG, Besmer P, Lyden D, Moore MA, Werb Z, Rafii S. Recruitment of stem and progenitor cells from the bone marrow niche requires MMP-9 mediated release of kit-ligand. Cell 2002;109:625-37.
75. Levesque JP, Liu F, Simmons PJ, Betsuyaku T, Senior RM, Pham C, Link DC. Characterization of hematopoietic progenitor mobilization in protease-deficient mice. Blood 2004;104:65-72.
76. Harada M, Qin Y, Takano H, Minamino T, Zou Y, Toko H, Ohtsuka M, Matsuura K, Sano M, Nishi J, Iwanaga K, Akazawa H, Kunieda T, Zhu W, Hasegawa H, Kunisada K, Nagai T, Nakaya H, Yamauchi-Takihara K, Komuro I. G-CSF prevents cardiac remodeling after myocardial infarction by activating the Jak-Stat pathway in cardiomyocytes. Nat Med 2005;11:305-11.

77. Hilfiker-Kleiner D, Hilfiker A, Fuchs M, Kaminski K, Schaefer A, Schieffer B, Hillmer A, Schmiedl A, Ding Z, Podewski E, Podewski E, Poli V, Schneider MD, Schulz R, Park JK, Wollert KC, Drexler H. Signal transducer and activator of transcription 3 is required for myocardial capillary growth, control of interstitial matrix deposition, and heart protection from ischemic injury. Circ Res 2004;95:187-95.
78. Yamauchi-Takihara K, Kishimoto T. A novel role for STAT3 in cardiac remodeling. Trends Cardiovasc Med 2000;10:298-303.
79. Ueda K, Takano H, Hasegawa H, Niitsuma Y, Qin Y, Ohtsuka M, Komuro I. Granulocyte colony stimulating factor directly inhibits myocardial ischemia-reperfusion injury through Akt-endothelial NO synthase pathway. Arterioscler Thromb Vasc Biol 2006;26:e108-e113.
80. Boneberg EM, Hareng L, Gantner F, Wendel A, Hartung T. Human monocytes express functional receptors for granulocyte colony-stimulating factor that mediate suppression of monokines and interferon-gamma. Blood 2000;95:270-6.
81. Hartung T. Anti-inflammatory effects of granulocyte colony-stimulating factor. Curr Opin Hematol 1998;5:221-5.
82. Misao Y, Takemura G, Arai M, Ohno T, Onogi H, Takahashi T, Minatoguchi S, Fujiwara T, Fujiwara H. Importance of recruitment of bone marrow-derived CXCR4+ cells in post-infarct cardiac repair mediated by G-CSF. Cardiovasc Res 2006;71:455-65.
83. Anderlini P, Przepiorka D, Seong D, Miller P, Sundberg J, Lichtiger B, Norfleet F, Chan KW, Champlin R, Korbling M. Clinical toxicity and laboratory effects of granulocyte-colony-stimulating factor (filgrastim) mobilization and blood stem cell apheresis from normal donors, and analysis of charges for the procedures. Transfusion 1996;36:590-5.
84. Cavallaro AM, Lilleby K, Majolino I, Storb R, Appelbaum FR, Rowley SD, Bensinger WI. Three to six year follow-up of normal donors who received recombinant human granulocyte colony-stimulating factor. Bone Marrow Transplant 2000;25:85-9.
85. Carmeliet P. Mechanisms of angiogenesis and arteriogenesis. Nat Med 2000;6:389-95.
86. Lancrin C, Sroczynska P, Stephenson C, Allen T, Kouskoff V, Lacaud G. The haemangioblast generates haematopoietic cells through a haemogenic endothelium stage. Nature 2009;457:892-5.
87. Ferguson JE, III, Kelley RW, Patterson C. Mechanisms of endothelial differentiation in embryonic vasculogenesis. Arterioscler Thromb Vasc Biol 2005;25:2246-54.
88. McGrath KE, Koniski AD, Maltby KM, McGann JK, Palis J. Embryonic expression and function of the chemokine SDF-1 and its receptor, CXCR4. Dev Biol 1999;213:442-56.
89. Cox CM, Poole TJ. Angioblast differentiation is influenced by the local environment: FGF-2 induces angioblasts and patterns vessel formation in the quail embryo. Dev Dyn 2000;218:371-82.
90. Carmeliet P, Ferreira V, Breier G, Pollefeyt S, Kieckens L, Gertsenstein M, Fahrig M, Vandenhoeck A, Harpal K, Eberhardt C, Declercq C, Pawling J, Moons L, Collen D, Risau W, Nagy A. Abnormal blood vessel development and lethality in embryos lacking a single VEGF allele. Nature 1996;380:435-9.
91. Tomanek RJ, Christensen LP, Simons M, Murakami M, Zheng W, Schatteman GC. Embryonic coronary vasculogenesis and angiogenesis are regulated by interactions between multiple FGFs and VEGF and are influenced by mesenchymal stem cells. Dev Dyn 2010;239:3182-91.
92. Takahashi T, Kalka C, Masuda H, Chen D, Silver M, Kearney M, Magner M, Isner JM, Asahara T. Ischemia- and cytokine-induced mobilization of bone marrow-derived endothelial progenitor cells for neovascularization. Nat Med 1999;5:434-8.
93. Asahara T, Takahashi T, Masuda H, Kalka C, Chen D, Iwaguro H, Inai Y, Silver M, Isner JM. VEGF contributes to postnatal neovascularization by mobilizing bone marrow-derived endothelial progenitor cells. EMBO J 1999;18:3964-72.
94. Hattori K, Dias S, Heissig B, Hackett NR, Lyden D, Tateno M, Hicklin DJ, Zhu Z, Witte L, Crystal RG, Moore MA, Rafii S. Vascular endothelial growth factor and angiopoietin-1 stimulate postnatal hematopoiesis by recruitment of vasculogenic and hematopoietic stem cells. J Exp Med 2001;193:1005-14.
95. Ceradini DJ, Kulkarni AR, Callaghan MJ, Tepper OM, Bastidas N, Kleinman ME, Capla JM, Galiano RD, Levine JP, Gurtner GC. Progenitor cell trafficking is regulated by hypoxic gradients through HIF-1 induction of SDF-1. Nat Med 2004;10:858-64.
96. Kaelin WG, Jr., Ratcliffe PJ. Oxygen sensing by metazoans: the central role of the HIF hydroxylase pathway. Mol Cell 2008;30:393-402.
97. Kelly BD, Hackett SF, Hirota K, Oshima Y, Cai Z, Berg-Dixon S, Rowan A, Yan Z, Campochiaro PA, Semenza GL. Cell type-specific regulation of angiogenic growth factor gene expression and induction of angiogenesis in nonischemic tissue by a constitutively active form of hypoxia-inducible factor 1. Circ Res 2003;93:1074-81.
98. Ruhrberg C, Gerhardt H, Golding M, Watson R, Ioannidou S, Fujisawa H, Betsholtz C, Shima DT. Spatially restricted patterning cues provided by heparin-binding VEGF-A control blood vessel branching morphogenesis. Genes Dev 2002;16:2684-98.
99. Gerhardt H, Golding M, Fruttiger M, Ruhrberg C, Lundkvist A, Abramsson A, Jeltsch M, Mitchell C, Alitalo K, Shima D, Betsholtz C. VEGF guides angiogenic sprouting utilizing endothelial tip cell filopodia. J Cell Biol 2003;161:1163-77.
100. Hellstrom M, Phng LK, Hofmann JJ, Wallgard E, Coultas L, Lindblom P, Alva J, Nilsson AK, Karlsson L, Gaiano N, Yoon K, Rossant J, Iruela-Arispe ML, Kalen M, Gerhardt H, Betsholtz C. Dll4 signalling through Notch1 regulates formation of tip cells during angiogenesis. Nature 2007;445:776-80.
101. Yana I, Sagara H, Takaki S, Takatsu K, Nakamura K, Nakao K, Katsuki M, Taniguchi S, Aoki T, Sato H, Weiss SJ, Seiki M. Crosstalk between neovessels and mural cells directs the site-specific expression of MT1-MMP to endothelial tip cells. J Cell Sci 2007;120:1607-14.
102. Fantin A, Vieira JM, Gestri G, Denti L, Schwarz Q, Prykhozhij S, Peri F, Wilson SW, Ruhrberg C. Tissue macrophages act as cellular chaperones for vascular anastomosis downstream of VEGF-mediated endothelial tip cell induction. Blood 2010;116:829-40.
103. Demicheva E, Hecker M, Korff T. Stretch-induced activation of the transcription factor activator protein-1 controls monocyte chemoattractant protein-1 expression during arteriogenesis. Circ Res 2008;103:477-84.
104. Garcia-Cardena G, Comander J, Anderson KR, Blackman BR, Gimbrone MA, Jr. Biomechanical activation of vascular endothelium as a determinant of its functional phenotype. Proc Natl Acad Sci U S A 2001;98:4478-85.
105. Lee CW, Stabile E, Kinnaird T, Shou M, Devaney JM, Epstein SE, Burnett MS. Temporal patterns of gene expression after acute hindlimb ischemia in mice: insights into the genomic

program for collateral vessel development. J Am Coll Cardiol 2004;43:474-82.
106. Scholz D, Ito W, Fleming I, Deindl E, Sauer A, Wiesnet M, Busse R, Schaper J, Schaper W. Ultrastructure and molecular histology of rabbit hind-limb collateral artery growth (arteriogenesis). Virchows Arch 2000;436:257-70.
107. Cai W, Vosschulte R, fsah-Hedjri A, Koltai S, Kocsis E, Scholz D, Kostin S, Schaper W, Schaper J. Altered balance between extracellular proteolysis and antiproteolysis is associated with adaptive coronary arteriogenesis. J Mol Cell Cardiol 2000;32:997-1011.
108. Wu S, Wu X, Zhu W, Cai WJ, Schaper J, Schaper W. Immunohistochemical study of the growth factors, aFGF, bFGF, PDGF-AB, VEGF-A and its receptor (Flk-1) during arteriogenesis. Mol Cell Biochem 2010;343:223-9.
109. Kinnaird T, Stabile E, Burnett MS, Lee CW, Barr S, Fuchs S, Epstein SE. Marrow-derived stromal cells express genes encoding a broad spectrum of arteriogenic cytokines and promote in vitro and in vivo arteriogenesis through paracrine mechanisms. Circ Res 2004;94:678-85.
110. Hoefer IE, van RN, Buschmann IR, Piek JJ, Schaper W. Time course of arteriogenesis following femoral artery occlusion in the rabbit. Cardiovasc Res 2001;49:609-17.
111. Behm CZ, Kaufmann BA, Carr C, Lankford M, Sanders JM, Rose CE, Kaul S, Lindner JR. Molecular imaging of endothelial vascular cell adhesion molecule-1 expression and inflammatory cell recruitment during vasculogenesis and ischemia-mediated arteriogenesis. Circulation 2008;117:2902-11.
112. Soehnlein O, Zernecke A, Eriksson EE, Rothfuchs AG, Pham CT, Herwald H, Bidzhekov K, Rottenberg ME, Weber C, Lindbom L. Neutrophil secretion products pave the way for inflammatory monocytes. Blood 2008;112:1461-71.
113. Bergmann CE, Hoefer IE, Meder B, Roth H, van RN, Breit SM, Jost MM, Aharinejad S, Hartmann S, Buschmann IR. Arteriogenesis depends on circulating monocytes and macrophage accumulation and is severely depressed in op/op mice. J Leukoc Biol 2006;80:59-65.
114. Bakker EN, Matlung HL, Bonta P, de Vries CJ, van RN, Vanbavel E. Blood flow-dependent arterial remodelling is facilitated by inflammation but directed by vascular tone. Cardiovasc Res 2008;78:341-8.
115. Khmelewski E, Becker A, Meinertz T, Ito WD. Tissue resident cells play a dominant role in arteriogenesis and concomitant macrophage accumulation. Circ Res 2004;95:E56-E64.
116. Aicher A, Rentsch M, Sasaki K, Ellwart JW, Fandrich F, Siebert R, Cooke JP, Dimmeler S, Heeschen C. Nonbone marrow-derived circulating progenitor cells contribute to postnatal neovascularization following tissue ischemia. Circ Res 2007;100:581-9.
117. Tepper OM, Capla JM, Galiano RD, Ceradini DJ, Callaghan MJ, Kleinman ME, Gurtner GC. Adult vasculogenesis occurs through in situ recruitment, proliferation, and tubulization of circulating bone marrow-derived cells. Blood 2005;105:1068-77.
118. Reinecke H, Minami E, Zhu WZ, Laflamme MA. Cardiogenic differentiation and transdifferentiation of progenitor cells. Circ Res 2008;103:1058-71.
119. Copelan EA. Hematopoietic stem-cell transplantation. N Engl J Med 2006;354:1813-26.
120. Shpall EJ, Champlin R, Glaspy JA. Effect of CD34+ peripheral blood progenitor cell dose on hematopoietic recovery. Biol Blood Marrow Transplant 1998;4:84-92.
121. Rota M, Kajstura J, Hosoda T, Bearzi C, Vitale S, Esposito G, Iaffaldano G, Padin-Iruegas ME, Gonzalez A, Rizzi R, Small N, Muraski J, Alvarez R, Chen X, Urbanek K, Bolli R, Houser SR, Leri A, Sussman MA, Anversa P. Bone marrow cells adopt the cardiomyogenic fate in vivo. Proc Natl Acad Sci U S A 2007;104:17783-8.
122. 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.
123. Richardson MR, Yoder MC. Endothelial progenitor cells: Quo Vadis? J Mol Cell Cardiol 2011;50:266-72.
124. Timmermans F, Van HF, De SM, Raedt R, Plasschaert F, De Buyzere ML, Gillebert TC, Plum J, Vandekerckhove B. Endothelial outgrowth cells are not derived from CD133+ cells or CD45+ hematopoietic precursors. Arterioscler Thromb Vasc Biol 2007;27:1572-9.
125. Massa M, Rosti V, Ferrario M, Campanelli R, Ramajoli I, Rosso R, De Ferrari GM, Ferlini M, Goffredo L, Bertoletti A, Klersy C, Pecci A, Moratti R, Tavazzi L. Increased circulating hematopoietic and endothelial progenitor cells in the early phase of acute myocardial infarction. Blood 2005;105:199-206.
126. Kunz GA, Liang G, Cuculi F, Gregg D, Vata KC, Shaw LK, Goldschmidt-Clermont PJ, Dong C, Taylor DA, Peterson ED. Circulating endothelial progenitor cells predict coronary artery disease severity. Am Heart J 2006;152:190-5.
127. Wang CH, Verma S, Hsieh IC, Chen YJ, Kuo LT, Yang NI, Wang SY, Wu MY, Hsu CM, Cheng CW, Cherng WJ. Enalapril increases ischemia-induced endothelial progenitor cell mobilization through manipulation of the CD26 system. J Mol Cell Cardiol 2006;41:34-43.
128. Vasa M, Fichtlscherer S, Adler K, Aicher A, Martin H, Zeiher AM, Dimmeler S. Increase in circulating endothelial progenitor cells by statin therapy in patients with stable coronary artery disease. Circulation 2001;103:2885-90.
129. Shi Q, Rafii S, Wu MH, Wijelath ES, Yu C, Ishida A, Fujita Y, Kothari S, Mohle R, Sauvage LR, Moore MA, Storb RF, Hammond WP. Evidence for circulating bone marrow-derived endothelial cells. Blood 1998;92:362-7.
130. Urbich C, Aicher A, Heeschen C, Dernbach E, Hofmann WK, Zeiher AM, Dimmeler S. Soluble factors released by endothelial progenitor cells promote migration of endothelial cells and cardiac resident progenitor cells. J Mol Cell Cardiol 2005;39:733-42.
131. Badorff C, Brandes RP, Popp R, Rupp S, Urbich C, Aicher A, Fleming I, Busse R, Zeiher AM, Dimmeler S. Transdifferentiation of blood-derived human adult endothelial progenitor cells into functionally active cardiomyocytes. Circulation 2003;107:1024-32.
132. Koyanagi M, Urbich C, Chavakis E, Hoffmann J, Rupp S, Badorff C, Zeiher AM, Starzinski-Powitz A, Haendeler J, Dimmeler S. Differentiation of circulating endothelial progenitor cells to a cardiomyogenic phenotype depends on E-cadherin. FEBS Lett 2005;579:6060-6.
133. Gruh I, Beilner J, Blomer U, Schmiedl A, Schmidt-Richter I, Kruse ML, Haverich A, Martin U. No evidence of transdifferentiation of human endothelial progenitor cells into cardiomyocytes after coculture with neonatal rat cardiomyocytes. Circulation 2006;113:1326-34.
134. Friedenstein AJ, Chailakhyan RK, Latsinik NV, Panasyuk AF, Keiliss-Borok IV. Stromal cells responsible for transferring the microenvironment of the hemopoietic tissues. Cloning in vi-

tro and retransplantation in vivo. Transplantation 1974;17:331-40.
135. Jiang Y, Jahagirdar BN, Reinhardt RL, Schwartz RE, Keene CD, Ortiz-Gonzalez XR, Reyes M, Lenvik T, Lund T, Blackstad M, Du J, Aldrich S, Lisberg A, Low WC, Largaespada DA, Verfaillie CM. Pluripotency of mesenchymal stem cells derived from adult marrow. Nature 2002;418:41-9.
136. Reyes M, Verfaillie CM. Characterization of multipotent adult progenitor cells, a subpopulation of mesenchymal stem cells. Ann N Y Acad Sci 2001;938:231-3.
137. Porada CD, meida-Porada G. Mesenchymal stem cells as therapeutics and vehicles for gene and drug delivery. Adv Drug Deliv Rev 2010;62:1156-66.
138. Crisan M, Yap S, Casteilla L, Chen CW, Corselli M, Park TS, Andriolo G, Sun B, Zheng B, Zhang L, Norotte C, Teng PN, Traas J, Schugar R, Deasy BM, Badylak S, Buhring HJ, Giacobino JP, Lazzari L, Huard J, Peault B. A perivascular origin for mesenchymal stem cells in multiple human organs. Cell Stem Cell 2008;3:301-13.
139. Corselli M, Chen CW, Crisan M, Lazzari L, Peault B. Perivascular ancestors of adult multipotent stem cells. Arterioscler Thromb Vasc Biol 2010;30:1104-9.
140. Rojewski M, Weber B, Schrezenmeier H. Phenotypic Characterization of Mesenchymal Stem Cells from Various Tissues. Transfus Med Hemother 2008;35:168-84.
141. Dominici M, Le BK, Mueller I, Slaper-Cortenbach I, Marini F, Krause D, Deans R, Keating A, Prockop D, Horwitz E. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. Cytotherapy 2006;8:315-7.
142. Horwitz EM, Le BK, Dominici M, Mueller I, Slaper-Cortenbach I, Marini FC, Deans RJ, Krause DS, Keating A. Clarification of the nomenclature for MSC: The International Society for Cellular Therapy position statement. Cytotherapy 2005;7:393-5.
143. Wu X, Huang L, Zhou Q, Song Y, Li A, Jin J, Cui B. Mesenchymal stem cells participating in ex vivo endothelium repair and its effect on vascular smooth muscle cells growth. Int J Cardiol 2005;105:274-82.
144. Oswald J, Boxberger S, Jorgensen B, Feldmann S, Ehninger G, Bornhauser M, Werner C. Mesenchymal stem cells can be differentiated into endothelial cells in vitro. Stem Cells 2004;22:377-84.
145. Ball SG, Shuttleworth AC, Kielty CM. Direct cell contact influences bone marrow mesenchymal stem cell fate. Int J Biochem Cell Biol 2004;36:714-27.
146. Toma C, Pittenger MF, Cahill KS, Byrne BJ, Kessler PD. Human mesenchymal stem cells differentiate to a cardiomyocyte phenotype in the adult murine heart. Circulation 2002;105:93-8.
147. Tokcaer-Keskin Z, Akar AR, yaloglu-Butun F, Terzioglu-Kara E, Durdu S, Ozyurda U, Ugur M, Akcali KC. Timing of induction of cardiomyocyte differentiation for in vitro cultured mesenchymal stem cells: a perspective for emergencies. Can J Physiol Pharmacol 2009;87:143-50.
148. Dai W, Hale SL, Martin BJ, Kuang JQ, Dow JS, Wold LE, Kloner RA. Allogeneic Mesenchymal Stem Cell Transplantation in Postinfarcted Rat Myocardium: Short- and Long-Term Effects. Circulation 2005;112:214-23.
149. Iso Y, Spees JL, Serrano C, Bakondi B, Pochampally R, Song YH, Sobel BE, Delafontaine P, Prockop DJ. Multipotent human stromal cells improve cardiac function after myocardial infarction in mice without long-term engraftment. Biochem Biophys Res Commun 2007;354:700-6.
150. Sadat S, Gehmert S, Song YH, Yen Y, Bai X, Gaiser S, Klein H, Alt E. The cardioprotective effect of mesenchymal stem cells is mediated by IGF-I and VEGF. Biochem Biophys Res Commun 2007;363:674-9.
151. Honczarenko M, Le Y, Swierkowski M, Ghiran I, Glodek AM, Silberstein LE. Human bone marrow stromal cells express a distinct set of biologically functional chemokine receptors. Stem Cells 2006;24:1030-41.
152. Von L, I, Notohamiprodjo M, Wechselberger A, Peters C, Henger A, Seliger C, Djafarzadeh R, Huss R, Nelson PJ. Human adult CD34- progenitor cells functionally express the chemokine receptors CCR1, CCR4, CCR7, CXCR5, and CCR10 but not CXCR4. Stem Cells Dev 2005;14:329-36.
153. Barbash IM, Chouraqui P, Baron J, Feinberg MS, Etzion S, Tessone A, Miller L, Guetta E, Zipori D, Kedes LH, Kloner RA, Leor J. Systemic delivery of bone marrow-derived mesenchymal stem cells to the infarcted myocardium: feasibility, cell migration, and body distribution. Circulation 2003;108:863-8.
154. Morigi M, Imberti B, Zoja C, Corna D, Tomasoni S, Abbate M, Rottoli D, Angioletti S, Benigni A, Perico N, Alison M, Remuzzi G. Mesenchymal stem cells are renotropic, helping to repair the kidney and improve function in acute renal failure. J Am Soc Nephrol 2004;15:1794-804.
155. Granero-Molto F, Weis JA, Miga MI, Landis B, Myers TJ, O'Rear L, Longobardi L, Jansen ED, Mortlock DP, Spagnoli A. Regenerative effects of transplanted mesenchymal stem cells in fracture healing. Stem Cells 2009;27:1887-98.
156. Ruster B, Gottig S, Ludwig RJ, Bistrian R, Muller S, Seifried E, Gille J, Henschler R. Mesenchymal stem cells display coordinated rolling and adhesion behavior on endothelial cells. Blood 2006;108:3938-44.
157. Cheng Z, Ou L, Zhou X, Li F, Jia X, Zhang Y, Liu X, Li Y, Ward CA, Melo LG, Kong D. Targeted migration of mesenchymal stem cells modified with CXCR4 gene to infarcted myocardium improves cardiac performance. Mol Ther 2008;16:571-9.
158. Cho HH, Kyoung KM, Seo MJ, Kim YJ, Bae YC, Jung JS. Overexpression of CXCR4 increases migration and proliferation of human adipose tissue stromal cells. Stem Cells Dev 2006;15:853-64.
159. Ip JE, Wu Y, Huang J, Zhang L, Pratt RE, Dzau VJ. Mesenchymal stem cells use integrin beta1 not CXC chemokine receptor 4 for myocardial migration and engraftment. Mol Biol Cell 2007;18:2873-82.
160. Schenk S, Mal N, Finan A, Zhang M, Kiedrowski M, Popovic Z, McCarthy PM, Penn MS. Monocyte chemotactic protein-3 is a myocardial mesenchymal stem cell homing factor. Stem Cells 2007;25:245-51.
161. Silva GV, Litovsky S, Assad JA, Sousa AL, Martin BJ, Vela D, Coulter SC, Lin J, Ober J, Vaughn WK, Branco RV, Oliveira EM, He R, Geng YJ, Willerson JT, Perin EC. Mesenchymal stem cells differentiate into an endothelial phenotype, enhance vascular density, and improve heart function in a canine chronic ischemia model. Circulation 2005;111:150-6.
162. Shake JG, Gruber PJ, Baumgartner WA, Senechal G, Meyers J, Redmond JM, Pittenger MF, Martin BJ. Mesenchymal stem cell implantation in a swine myocardial infarct model: engraftment and functional effects. Ann Thorac Surg 2002;73:1919-25.
163. Amado LC, Saliaris AP, Schuleri KH, St JM, Xie JS, Cattaneo S, Durand DJ, Fitton T, Kuang JQ, Stewart G, Lehrke S, Baumgartner WW, Martin BJ, Heldman AW, Hare JM. Cardiac re-

pair with intramyocardial injection of allogeneic mesenchymal stem cells after myocardial infarction. Proc Natl Acad Sci U S A 2005;102:11474-9.
164. Miyahara Y, Nagaya N, Kataoka M, Yanagawa B, Tanaka K, Hao H, Ishino K, Ishida H, Shimizu T, Kangawa K, Sano S, Okano T, Kitamura S, Mori H. Monolayered mesenchymal stem cells repair scarred myocardium after myocardial infarction. Nat Med 2006;12:459-65.
165. Ziegelhoeffer T, Fernandez B, Kostin S, Heil M, Voswinckel R, Helisch A, Schaper W. Bone marrow-derived cells do not incorporate into the adult growing vasculature. Circ Res 2004;94:230-8.
166. Lee RH, Pulin AA, Seo MJ, Kota DJ, Ylostalo J, Larson BL, Semprun-Prieto L, Delafontaine P, Prockop DJ. Intravenous hMSCs improve myocardial infarction in mice because cells embolized in lung are activated to secrete the anti-inflammatory protein TSG-6. Cell Stem Cell 2009;5:54-63.
167. He Q, Wan C, Li G. Concise review: multipotent mesenchymal stromal cells in blood. Stem Cells 2007;25:69-77.
168. Kuznetsov SA, Mankani MH, Gronthos S, Satomura K, Bianco P, Robey PG. Circulating skeletal stem cells. J Cell Biol 2001;153:1133-40.
169. Tondreau T, Meuleman N, Delforge A, Dejeneffe M, Leroy R, Massy M, Mortier C, Bron D, Lagneaux L. Mesenchymal stem cells derived from CD133-positive cells in mobilized peripheral blood and cord blood: proliferation, Oct4 expression, and plasticity. Stem Cells 2005;23:1105-12.
170. Fernandez M, Simon V, Herrera G, Cao C, Del FH, Minguell JJ. Detection of stromal cells in peripheral blood progenitor cell collections from breast cancer patients. Bone Marrow Transplant 1997;20:265-71.
171. Wexler SA, Donaldson C, ning-Kendall P, Rice C, Bradley B, Hows JM. Adult bone marrow is a rich source of human mesenchymal /`stem/' cells but umbilical cord and mobilized adult blood are not. Br J Haematol 2003;121:368-74.
172. Lazarus HM, Haynesworth SE, Gerson SL, Caplan AI. Human bone marrow-derived mesenchymal stromal (progenitor cells MPCs) cannot be recovered from peripheral blood progenitor cell collections. J Hematother 1997;6:447-55.
173. Kassis I, Zangi L, Rivkin R, Levdansky L, Samuel S, Marx G, Gorodetsky R. Isolation of mesenchymal stem cells from G-CSF-mobilized human peripheral blood using fibrin microbeads. Bone Marrow Transplant 2006;37:967-76.
174. Kawamoto A, Gwon HC, Iwaguro H, Yamaguchi JI, Uchida S, Masuda H, Silver M, Ma H, Kearney M, Isner JM, Asahara T. Therapeutic potential of ex vivo expanded endothelial progenitor cells for myocardial ischemia. Circulation 2001;103:634-7.
175. Oswald J, Boxberger S, Jorgensen B, Feldmann S, Ehninger G, Bornhauser M, Werner C. Mesenchymal stem cells can be differentiated into endothelial cells in vitro. Stem Cells 2004;22:377-84.
176. Yoon CH, Koyanagi M, Iekushi K, Seeger F, Urbich C, Zeiher AM, Dimmeler S. Mechanism of improved cardiac function after bone marrow mononuclear cell therapy: role of cardiovascular lineage commitment. Circulation 2010;121:2001-11.
177. Gnecchi M, Zhang Z, Ni A, Dzau VJ. Paracrine mechanisms in adult stem cell signaling and therapy. Circ Res 2008;103:1204-19.
178. Gnecchi M, He H, Liang OD, Melo LG, Morello F, Mu H, Noiseux N, Zhang L, Pratt RE, Ingwall JS, Dzau VJ. Paracrine action accounts for marked protection of ischemic heart by Akt-modified mesenchymal stem cells. Nat Med 2005;11:367-8.
179. Takahashi M, Li TS, Suzuki R, Kobayashi T, Ito H, Ikeda Y, Matsuzaki M, Hamano K. Cytokines produced by bone marrow cells can contribute to functional improvement of the infarcted heart by protecting cardiomyocytes from ischemic injury. Am J Physiol Heart Circ Physiol 2006;291:H886-H893.
180. Maxeiner H, Krehbiehl N, Muller A, Woitasky N, Akinturk H, Muller M, Weigand MA, Abdallah Y, Kasseckert S, Schreckenberg R, Schluter KD, Wenzel S. New insights into paracrine mechanisms of human cardiac progenitor cells. Eur J Heart Fail 2010;12:730-7.
181. Kinnaird T, Stabile E, Burnett MS, Shou M, Lee CW, Barr S, Fuchs S, Epstein SE. Local delivery of marrow-derived stromal cells augments collateral perfusion through paracrine mechanisms. Circulation 2004;109:1543-9.
182. Shabbir A, Zisa D, Lin H, Mastri M, Roloff G, Suzuki G, Lee T. Activation of host tissue trophic factors through JAK-STAT3 signaling: a mechanism of mesenchymal stem cell-mediated cardiac repair. Am J Physiol Heart Circ Physiol 2010;299:H1428-H1438.
183. Lewis EF, Johnson PA, Johnson W, Collins C, Griffin L, Stevenson LW. Preferences for quality of life or survival expressed by patients with heart failure. J Heart Lung Transplant 2001;20:1016-24.
184. Sugeng L, Mor-Avi V, Weinert L, Niel J, Ebner C, Steringer-Mascherbauer R, Schmidt F, Galuschky C, Schummers G, Lang RM, Nesser HJ. Quantitative assessment of left ventricular size and function: side-by-side comparison of real-time three-dimensional echocardiography and computed tomography with magnetic resonance reference. Circulation 2006;114:654-61.
185. St John SM, Pfeffer MA, Plappert T, Rouleau JL, Moye LA, Dagenais GR, Lamas GA, Klein M, Sussex B, Goldman S, . Quantitative two-dimensional echocardiographic measurements are major predictors of adverse cardiovascular events after acute myocardial infarction. The protective effects of captopril. Circulation 1994;89:68-75.
186. Kjoller E, Kober L, Jorgensen S, Torp-Pedersen C. Long-term prognostic importance of hyperkinesia following acute myocardial infarction. TRACE Study Group. TRAndolapril Cardiac Evaluation. Am J Cardiol 1999;83:655-9.
187. Moller JE, Hillis GS, Oh JK, Reeder GS, Gersh BJ, Pellikka PA. Wall motion score index and ejection fraction for risk stratification after acute myocardial infarction. Am Heart J 2006;151:419-25.
188. Lang RM, Bierig M, Devereux RB, Flachskampf FA, Foster E, Pellikka PA, Picard MH, Roman MJ, Seward J, Shanewise JS, Solomon SD, Spencer KT, Sutton MS, Stewart WJ. Recommendations for chamber quantification: a report from the American Society of Echocardiography's Guidelines and Standards Committee and the Chamber Quantification Writing Group, developed in conjunction with the European Association of Echocardiography, a branch of the European Society of Cardiology. J Am Soc Echocardiogr 2005;18:1440-63.
189. McGowan JH, Cleland JG. Reliability of reporting left ventricular systolic function by echocardiography: a systematic review of 3 methods. Am Heart J 2003;146:388-97.
190. Faber TL, Cooke CD, Folks RD, Vansant JP, Nichols KJ, DePuey EG, Pettigrew RI, Garcia EV. Left ventricular function and perfusion from gated SPECT perfusion images: an integrated method. J Nucl Med 1999;40:650-9.

191. Nakajima K, Nishimura T. Inter-institution preference-based variability of ejection fraction and volumes using quantitative gated SPECT with 99mTc-tetrofosmin: a multicentre study involving 106 hospitals. Eur J Nucl Med Mol Imaging 2006;33:127-33.
192. Koch JA, Poll LW, Godehardt E, Korbmacher B, Modder U. Right and left ventricular volume measurements in an animal heart model in vitro: first experiences with cardiac MRI at 1.0 T. Eur Radiol 2000;10:455-8.
193. Møgelvang J, Stokholm KH, Saunamaki K, Reimer A, Stubgaard M, Thomsen C, Fritz-Hansen P, Henriksen O. Assessment of left ventricular volumes by magnetic resonance in comparison with radionuclide angiography, contrast angiography and echocardiography. Eur Heart J 1992;13:1677-83.
194. Florentine MS, Grosskreutz CL, Chang W, Hartnett JA, Dunn VD, Ehrhardt JC, Fleagle SR, Collins SM, Marcus ML, Skorton DJ. Measurement of left ventricular mass in vivo using gated nuclear magnetic resonance imaging. J Am Coll Cardiol 1986;8:107-12.
195. Mogelvang J, Lindvig K, Sondergaard L, Saunamaki K, Henriksen O. Reproducibility of cardiac volume measurements including left ventricular mass determined by MRI. Clin Physiol 1993;13:587-97.
196. Semelka RC, Tomei E, Wagner S, Mayo J, Caputo G, O'Sullivan M, Parmley WW, Chatterjee K, Wolfe C, Higgins CB. Interstudy reproducibility of dimensional and functional measurements between cine magnetic resonance studies in the morphologically abnormal left ventricle. Am Heart J 1990;119:1367-73.
197. Schwitter J, Nanz D, Kneifel S, Bertschinger K, Buchi M, Knusel PR, Marincek B, Luscher TF, von Schulthess GK. Assessment of myocardial perfusion in coronary artery disease by magnetic resonance: a comparison with positron emission tomography and coronary angiography. Circulation 2001;103:2230-5.
198. Ibrahim T, Nekolla SG, Schreiber K, Odaka K, Volz S, Mehilli J, Guthlin M, Delius W, Schwaiger M. Assessment of coronary flow reserve: comparison between contrast-enhanced magnetic resonance imaging and positron emission tomography. J Am Coll Cardiol 2002;39:864-70.
199. Nagel E, Klein C, Paetsch I, Hettwer S, Schnackenburg B, Wegscheider K, Fleck E. Magnetic resonance perfusion measurements for the noninvasive detection of coronary artery disease. Circulation 2003;108:432-7.
200. Sakuma H, Suzawa N, Ichikawa Y, Makino K, Hirano T, Kitagawa K, Takeda K. Diagnostic accuracy of stress first-pass contrast-enhanced myocardial perfusion MRI compared with stress myocardial perfusion scintigraphy. AJR Am J Roentgenol 2005;185:95-102.
201. Marckmann P. An epidemic outbreak of nephrogenic systemic fibrosis in a Danish hospital. Eur J Radiol 2008;66:187-90.
202. Kalka C, Masuda H, Takahashi T, Gordon R, Tepper O, Gravereaux E, Pieczek A, Iwaguro H, Hayashi SI, Isner JM, Asahara T. Vascular endothelial growth factor(165) gene transfer augments circulating endothelial progenitor cells in human subjects. Circ Res 2000;86:1198-202.
203. Tomita S, Li RK, Weisel RD, Mickle DA, Kim EJ, Sakai T, Jia ZQ. Autologous transplantation of bone marrow cells improves damaged heart function. Circulation 1999;100:II247-II256.
204. Sesti C, Hale SL, Lutzko C, Kloner RA. Granulocyte colony-stimulating factor and stem cell factor improve contractile reserve of the infarcted left ventricle independent of restoring muscle mass. J Am Coll Cardiol 2005;46:1662-9.
205. Kalka C, Masuda H, Takahashi T, Kalka-Moll WM, Silver M, Kearney M, Li T, Isner JM, Asahara T. Transplantation of ex vivo expanded endothelial progenitor cells for therapeutic neovascularization. Proc Natl Acad Sci U S A 2000;97:3422-7.
206. Uemura R, Xu M, Ahmad N, Ashraf M. Bone marrow stem cells prevent left ventricular remodeling of ischemic heart through paracrine signaling. Circ Res 2006;98:1414-21.
207. Orlic D, Kajstura J, Chimenti S, Limana F, Jakoniuk I, Quaini F, Nadal-Ginard B, Bodine DM, Leri A, Anversa P. Mobilized bone marrow cells repair the infarcted heart, improving function and survival. Proc Natl Acad Sci U S A 2001;98:10344-9.
208. Wang Y, Tägil K, Ripa RS, Nilsson JC, Carstensen S, Jørgensen E, Søndergaard L, Hesse B, Johnsen HE, Kastrup J. Effect of mobilization of bone marrow stem cells by granulocyte colony stimulating factor on clinical symptoms, left ventricular perfusion and function in patients with severe chronic ischemic heart disease. Int J Cardiol 2005;100:477-83.
209. Seiler C, Pohl T, Wustmann K, Hutter D, Nicolet PA, Windecker S, Eberli FR, Meier B. Promotion of collateral growth by granulocyte-macrophage colony-stimulating factor in patients with coronary artery disease: a randomized, double-blind, placebo-controlled study. Circulation 2001;104:2012-7.
210. Tateishi-Yuyama E, Matsubara H, Murohara T, Ikeda U, Shintani S, Masaki H, Amano K, Kishimoto Y, Yoshimoto K, Akashi H, Shimada K, Iwasaka T, Imaizumi T. 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.
211. Ripa RS, Wang Y, Jørgensen E, Johnsen HE, Grande P, Kastrup J. Safety of bone-marrow stem cell mobilisation induced by granulocyte-colony stimulating factor: Clinical 30 days blinded results from the stem cells in myocardial infarction (STEMMI) trial. Heart Drug 2005;5:177-82.
212. Hill JM, Syed MA, Arai AE, Powell TM, Paul JD, Zalos G, Read EJ, Khuu HM, Leitman SF, Horne M, Csako G, Dunbar CE, Waclawiw MA, Cannon RO, III. Outcomes and risks of granulocyte colony-stimulating factor in patients with coronary artery disease. J Am Coll Cardiol 2005;46:1643-8.
213. Boyle AJ, Whitbourn R, Schlicht S, Krum H, Kocher A, Nandurkar H, Bergmann S, Daniell M, O'Day J, Skerrett D, Haylock D, Gilbert RE, Itescu S. Intra-coronary high-dose CD34+ stem cells in patients with chronic ischemic heart disease: a 12-month follow-up. Int J Cardiol 2006;109:21-7.
214. Kovacic JC, Macdonald P, Feneley MP, Muller DWM, Freund J, Dodds A, Milliken S, Tao H, Itescu S, Moore J, Ma D, Graham RM. Safety and efficacy of consecutive cycles of granulocyte-colony stimulating factor, and an intracoronary CD133+ cell infusion in patients with chronic refractory ischemic heart disease: The G-CSF in Angina patients with IHD to stimulate Neovascularization (GAIN I) trial. Am Heart J 2008;156:954-63.
215. Losordo DW, Schatz RA, White CJ, Udelson JE, Veereshwarayya V, Durgin M, Poh KK, Weinstein R, Kearney M, Chaudhry M, Burg A, Eaton L, Heyd L, Thorne T, Shturman L, Hoffmeister P, Story K, Zak V, Dowling D, Traverse JH, Olson RE, Flanagan J, Sodano D, Murayama T, Kawamoto A, Kusano KF, Wollins J, Welt F, Shah P, Soukas P, Asahara T, Henry TD. Intramyocardial transplantation of autologous CD34+ stem cells for intractable angina: a phase I/IIa double-blind, randomized controlled trial. Circulation 2007;115:3165-72.

216. Kawamoto A, Murayama T, Kusano K, Ii M, Tkebuchava T, Shintani S, Iwakura A, Johnson I, von Samson P, Hanley A, Gavin M, Curry C, Silver M, Ma H, Kearney M, Losordo DW. Synergistic effect of bone marrow mobilization and vascular endothelial growth factor-2 gene therapy in myocardial ischemia. Circulation 2004;110:1398-405.
217. Shintani S, Kusano K, Ii M, Iwakura A, Heyd L, Curry C, Wecker A, Gavin M, Ma H, Kearney M, Silver M, Thorne T, Murohara T, Losordo DW. Synergistic effect of combined intramyocardial CD34+ cells and VEGF2 gene therapy after MI. Nat Clin Pract Cardiovasc Med 2006;3 Suppl 1:S123-S128.
218. Baldazzi F, Jørgensen E, Ripa RS, Kastrup J. Release of biomarkers of myocardial damage after direct intramyocardial injection of genes and stem cells via the percutaneous transluminal route. Eur Heart J 2008;29:1819-26.
219. Wang Y, Ripa RS, Jørgensen E, Hesse B, Mortensen S, Kastrup J. Mobilization of haematopoietic and non-haematopoietic cells by granulocyte-colony stimulating factor and vascular endothelial growth factor gene therapy in patients with stable severe coronary artery disease. Scand Cardiovasc J 2007;41:397-404.
220. Powell TM, Paul JD, Hill JM, Thompson M, Benjamin M, Rodrigo M, McCoy JP, Read EJ, Khuu HM, Leitman SF, Finkel T, Cannon RO, III. Granulocyte colony-stimulating factor mobilizes functional endothelial progenitor cells in patients with coronary artery disease. Arterioscler Thromb Vasc Biol 2005;25:296-301.
221. Takai H, Miyoshi A, Yamazaki M, Adachi K, Katagiri K, Arakawa H, Katsuyama K, Ito T, Fujii E, Hayashi S, Kato A, Suzuki M. Granulocyte colony-stimulating factor has no adverse effects on atherosclerotic lesions in high cholesterol-fed miniature Swine. J Vet Med Sci 2008;70:943-50.
222. Chih, Sharon S., Macdonald, Peter, McCrohon, Jane A., Ma, David, John, Moore, Law, Matthew, Feneley, Michael P., Herbert, Andrea, Kovacic, Jason C., and Graham, Robert M. Granulocyte-colony stimulating factor in angina patients with ischaemic heart diesase to stimulate neovascularisation (GAIN II trial). J Am Coll Cardiol 2010;55:A126.E1184 [Abstract]
223. Meier P, Gloekler S, de Marchi SF, Indermuehle A, Rutz T, Traupe T, Steck H, Vogel R, Seiler C. Myocardial salvage through coronary collateral growth by granulocyte colony-stimulating factor in chronic coronary artery disease: a controlled randomized trial. Circulation 2009;120:1355-63.
224. Goetze JP, Yongzhong W, Rehfeld JF, Jørgensen E, Kastrup J. Coronary angiography transiently increases plasma pro-B-type natriuretic peptide. Eur Heart J 2004;25:759-64.
225. Wang Y, Gabrielsen A, Lawler PR, Paulsson-Berne G, Steinbruchel DA, Hansson GK, Kastrup J. Myocardial gene expression of angiogenic factors in human chronic ischemic myocardium: influence of acute ischemia/cardioplegia and reperfusion. Microcirculation 2006;13:187-97.
226. Wynn RF, Hart CA, Corradi-Perini C, O'Neill L, Evans CA, Wraith JE, Fairbairn LJ, Bellantuono I. A small proportion of mesenchymal stem cells strongly expresses functionally active CXCR4 receptor capable of promoting migration to bone marrow. Blood 2004;104:2643-5.
227. Rentrop KP, Cohen M, Blanke H, Phillips RA. Changes in collateral channel filling immediately after controlled coronary artery occlusion by an angioplasty balloon in human subjects. J Am Coll Cardiol 1985;5:587-92.
228. Werner GS, Ferrari M, Heinke S, Kuethe F, Surber R, Richartz BM, Figulla HR. Angiographic assessment of collateral connections in comparison with invasively determined collateral function in chronic coronary occlusions. Circulation 2003;107:1972-7.
229. van der MP, De Boer RA, White HL, van der SG, Hall AS, Voors AA, van Veldhuisen DJ. The VEGF +405 CC promoter polymorphism is associated with an impaired prognosis in patients with chronic heart failure: a MERIT-HF substudy. J Card Fail 2005;11:279-84.
230. Ray D, Mishra M, Ralph S, Read I, Davies R, Brenchley P. Association of the VEGF gene with proliferative diabetic retinopathy but not proteinuria in diabetes. Diabetes 2004;53:861-4.
231. Doi K, Noiri E, Nakao A, Fujita T, Kobayashi S, Tokunaga K. Functional polymorphisms in the vascular endothelial growth factor gene are associated with development of end-stage renal disease in males. J Am Soc Nephrol 2006;17:823-30.
232. Askari AT, Unzek S, Popovic ZB, Goldman CK, Forudi F, Kiedrowski M, Rovner A, Ellis SG, Thomas JD, DiCorleto PE, Topol EJ, Penn MS. Effect of stromal-cell-derived factor 1 on stem-cell homing and tissue regeneration in ischaemic cardiomyopathy. Lancet 2003;362:697-703.
233. Grunewald M, Avraham I, Dor Y, Bachar-Lustig E, Itin A, Jung S, Chimenti S, Landsman L, Abramovitch R, Keshet E. VEGF-induced adult neovascularization: recruitment, retention, and role of accessory cells. Cell 2006;124:175-89.
234. Peled A, Grabovsky V, Habler L, Sandbank J, renzana-Seisdedos F, Petit I, Ben-Hur H, Lapidot T, Alon R. The chemokine SDF-1 stimulates integrin-mediated arrest of CD34(+) cells on vascular endothelium under shear flow. J Clin Invest 1999;104:1199-211.
235. De FE, Porcelli D, Torella AR, Straino S, Iachininoto MG, Orlandi A, Truffa S, Biglioli P, Napolitano M, Capogrossi MC, Pesce M. SDF-1 involvement in endothelial phenotype and ischemia-induced recruitment of bone marrow progenitor cells. Blood 2004;104:3472-82.
236. Peled A, Kollet O, Ponomaryov T, Petit I, Franitza S, Grabovsky V, Slav MM, Nagler A, Lider O, Alon R, Zipori D, Lapidot T. The chemokine SDF-1 activates the integrins LFA-1, VLA-4, and VLA-5 on immature human CD34(+) cells: role in transendothelial/stromal migration and engraftment of NOD/SCID mice. Blood 2000;95:3289-96.
237. Chavakis E, Aicher A, Heeschen C, Sasaki K, Kaiser R, El MN, Urbich C, Peters T, Scharffetter-Kochanek K, Zeiher AM, Chavakis T, Dimmeler S. Role of beta2-integrins for homing and neovascularization capacity of endothelial progenitor cells. J Exp Med 2005;201:63-72.
238. Urbich C, Heeschen C, Aicher A, Sasaki K, Bruhl T, Farhadi MR, Vajkoczy P, Hofmann WK, Peters C, Pennacchio LA, Abolmaali ND, Chavakis E, Reinheckel T, Zeiher AM, Dimmeler S. Cathepsin L is required for endothelial progenitor cell-induced neovascularization. Nat Med 2005;11:206-13.
239. van Weel V, Seghers L, de Vries MR, Kuiper EJ, Schlingemann RO, Bajema IM, Lindeman JH, is-van Diemen PM, van H, V, van Bockel JH, Quax PH. Expression of vascular endothelial growth factor, stromal cell-derived factor-1, and CXCR4 in human limb muscle with acute and chronic ischemia. Arterioscler Thromb Vasc Biol 2007;27:1426-32.
240. Heeschen C, Lehmann R, Honold J, Assmus B, Aicher A, Walter DH, Martin H, Zeiher AM, Dimmeler S. Profoundly reduced neovascularization capacity of bone marrow mononuclear cells derived from patients with chronic ischemic heart disease. Circulation 2004;109:1615-22.

241. Li TS, Kubo M, Ueda K, Murakami M, Ohshima M, Kobayashi T, Tanaka T, Shirasawa B, Mikamo A, Hamano K. Identification of risk factors related to poor angiogenic potency of bone marrow cells from different patients. Circulation 2009;120:S255-S261.
242. Friis T, Haack-Sorensen M, Hansen SK, Hansen L, Bindslev L, Kastrup J. Comparison of Mesenchymal Stromal Cells From Young Healthy Donors and Patients With Severe Chronic Coronary Artery Disease. Scand J Clin Lab Invest In press 2011.
243. Suuronen EJ, Hazra S, Zhang P, Vincent R, Kumarathasan P, Zhang Y, Price J, Chan V, Sellke FW, Mesana TG, Veinot JP, Ruel M. Impairment of human cell-based vasculogenesis in rats by hypercholesterolemia-induced endothelial dysfunction and rescue with L-arginine supplementation. J Thorac Cardiovasc Surg 2010;139:209-16.
244. Murohara T, Asahara T, Silver M, Bauters C, Masuda H, Kalka C, Kearney M, Chen D, Symes JF, Fishman MC, Huang PL, Isner JM. Nitric oxide synthase modulates angiogenesis in response to tissue ischemia. J Clin Invest 1998;101:2567-78.
245. Matsunaga T, Weihrauch DW, Moniz MC, Tessmer J, Warltier DC, Chilian WM. Angiostatin inhibits coronary angiogenesis during impaired production of nitric oxide. Circulation 2002;105:2185-91.
246. Walter DH, Haendeler J, Reinhold J, Rochwalsky U, Seeger F, Honold J, Hoffmann J, Urbich C, Lehmann R, renzana-Seisdesdos F, Aicher A, Heeschen C, Fichtlscherer S, Zeiher AM, Dimmeler S. Impaired CXCR4 signaling contributes to the reduced neovascularization capacity of endothelial progenitor cells from patients with coronary artery disease. Circ Res 2005;97:1142-51.
247. Honold J, Lehmann R, Heeschen C, Walter DH, Assmus B, Sasaki K, Martin H, Haendeler J, Zeiher AM, Dimmeler S. Effects of granulocyte colony stimulating factor on functional activities of endothelial progenitor cells in patients with chronic ischemic heart disease. Arterioscler Thromb Vasc Biol 2006;26:2238-43.
248. Brunner S, Huber BC, Fischer R, Groebner M, Hacker M, David R, Zaruba MM, Vallaster M, Rischpler C, Wilke A, Gerbitz A, Franz WM. G-CSF treatment after myocardial infarction: impact on bone marrow-derived vs cardiac progenitor cells. Exp Hematol 2008;36:695-702.
249. Stein A, Zohlnhofer D, Pogatsa-Murray G, von WJ, Steppich BA, Schomig A, Kastrati A, Ott I. Expression of CXCR4, VLA-1, LFA-3 and transducer of ERB in G-CSF-mobilised progenitor cells in acute myocardial infarction. Thromb Haemost 2010;103:638-43.
250. Suarez de LJ, Torres A, Herrera I, Pan M, Romero M, Pavlovic D, Segura J, Ojeda S, Sanchez J, Lopez RF, Medina A. [Effects of stem-cell mobilization with recombinant human granulocyte colony stimulating factor in patients with percutaneously revascularized acute anterior myocardial infarction]. Rev Esp Cardiol 2005;58:253-61.
251. Kuethe F, Figulla HR, Herzau M, Voth M, Fritzenwanger M, Opfermann T, Pachmann K, Krack A, Sayer HG, Gottschild D, Werner GS. Treatment with granulocyte colony-stimulating factor for mobilization of bone marrow cells in patients with acute myocardial infarction. Am Heart J 2005;150:115.
252. Valgimigli M, Rigolin GM, Cittanti C, Malagutti P, Curello S, Percoco G, Bugli AM, Della PM, Bragotti LZ, Ansani L, Mauro E, Lanfranchi A, Giganti M, Feggi L, Castoldi G, Ferrari R. Use of granulocyte-colony stimulating factor during acute myocardial infarction to enhance bone marrow stem cell mobilization in humans: clinical and angiographic safety profile. Eur Heart J 2005;26:1838-45.
253. Leone AM, Galiuto L, Garramone B, Rutella S, Giannico MB, Brugaletta S, Perfetti M, Liuzzo G, Porto I, Burzotta F, Niccoli G, Biasucci LM, Leone G, Rebuzzi AG, Crea F. Usefulness of granulocyte colony-stimulating factor in patients with a large anterior wall acute myocardial infarction to prevent left ventricular remodeling (the rigenera study). Am J Cardiol 2007;100:397-403.
254. Kang HJ, Kim HS, Koo BK, Kim YJ, Lee D, Sohn DW, Oh BH, Park YB. Intracoronary infusion of the mobilized peripheral blood stem cell by G-CSF is better than mobilization alone by G-CSF for improvement of cardiac function and remodeling: 2-year follow-up results of the Myocardial Regeneration and Angiogenesis in Myocardial Infarction with G-CSF and Intra-Coronary Stem Cell Infusion (MAGIC Cell) 1 trial. Am Heart J 2007;153:237-8.
255. Takano H, Hasegawa H, Kuwabara Y, Nakayama T, Matsuno K, Miyazaki Y, Yamamoto M, Fujimoto Y, Okada H, Okubo S, Fujita M, Shindo S, Kobayashi Y, Komiyama N, Takekoshi N, Imai K, Himi T, Ishibashi I, Komuro I. Feasibility and safety of granulocyte colony-stimulating factor treatment in patients with acute myocardial infarction. Int J Cardiol 2007;122:41-7.
256. Ince H, Petzsch M, Kleine HD, Schmidt H, Rehders T, Korber T, Schumichen C, Freund M, Nienaber CA. Preservation from left ventricular remodeling by front-integrated revascularization and stem cell liberation in evolving acute myocardial infarction by use of granulocyte-colony-stimulating factor (FIRSTLINE-AMI). Circulation 2005;112:3097-106.
257. Ellis SG, Penn MS, Bolwell B, Garcia M, Chacko M, Wang T, Brezina KJ, McConnell G, Topol EJ. Granulocyte colony stimulating factor in patients with large acute myocardial infarction: results of a pilot dose-escalation randomized trial. Am Heart J 2006;152:1051-14.
258. Zohlnhöfer D, Ott I, Mehilli J, Schomig K, Michalk F, Ibrahim T, Meisetschlager G, von Wedel J, Bollwein H, Seyfarth M, Dirschinger J, Schmitt C, Schwaiger M, Kastrati A, Schomig A. Stem cell mobilization by granulocyte colony-stimulating factor in patients with acute myocardial infarction: a randomized controlled trial. JAMA 2006;295:1003-10.
259. Engelmann MG, Theiss HD, Hennig-Theiss C, Huber A, Wintersperger BJ, Werle-Ruedinger AE, Schoenberg SO, Steinbeck G, Franz WM. Autologous bone marrow stem cell mobilization induced by granulocyte colony-stimulating factor after subacute ST-segment elevation myocardial infarction undergoing late revascularization: final results from the G-CSF-STEMI (Granulocyte Colony-Stimulating Factor ST-Segment Elevation Myocardial Infarction) trial. J Am Coll Cardiol 2006;48:1712-21.
260. Baks T, van Geuns RJ, Biagini E, Wielopolski P, Mollet NR, Cademartiri F, van der Giessen WJ, Krestin GP, Serruys PW, Duncker DJ, de Feyter PJ. Effects of primary angioplasty for acute myocardial infarction on early and late infarct size and left ventricular wall characteristics. J Am Coll Cardiol 2006;47:40-4.
261. Zohlnhöfer D, Dibra A, Koppara T, de WA, Ripa RS, Kastrup J, Valgimigli M, Schomig A, Kastrati A. Stem cell mobilization by granulocyte colony-stimulating factor for myocardial recovery after acute myocardial infarction: a meta-analysis. J Am Coll Cardiol 2008;51:1429-37.
262. Ripa, R. S., Jørgensen, E., and Kastrup, J. Risk of Adverse Events at 5-years Follow-up after Cell Mobilization with Granulocyte-Colony Stimulating Factor After Acute ST-Elevation

Myocardial Infarction. Circulation 2010;122:A15055 [Abstract]
263. Kang HJ, Kim HS, Zhang SY, Park KW, Cho HJ, Koo BK, Kim YJ, Soo LD, Sohn DW, Han KS, Oh BH, Lee MM, Park YB. Effects of intracoronary infusion of peripheral blood stem-cells 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.
264. Steinwender C, Hofmann R, Kammler J, Kypta A, Pichler R, Maschek W, Schuster G, Gabriel C, Leisch F. Effects of peripheral blood stem cell mobilization with granulocyte-colony stimulating factor and their transcoronary transplantation after primary stent implantation for acute myocardial infarction. Am Heart J 2006;151:1296-13.
265. Jørgensen E, Ripa RS, Helqvist S, Wang Y, Johnsen HE, Grande P, Kastrup J. In-stent neo-intimal hyperplasia after stem cell mobilization by granulocyte-colony stimulating factor Preliminary intracoronary ultrasound results from a double-blind randomized placebo-controlled study of patients treated with percutaneous coronary intervention for ST-elevation myocardial infarction (STEMMI Trial). Int J Cardiol 2006;111:174-7.
266. Jørgensen E, Baldazzi F, Ripa RS, Friis T, Wang Y, Helqvist S, Kastrup J. Instent neointimal hyperplasia after percutaneous intervention for ST-elevation myocardial infarction and treatment with granulocyte-colony stimulating factor. Results from the stem cells in myocardial infarction (STEMMI) trial. Int J Cardiol 2010;139:269-75.
267. Ince H, Valgimigli M, Petzsch M, de Lezo JS, Kuethe F, Dunkelmann S, Biondi-Zoccai G, Nienaber CA. Cardiovascular events and re-stenosis following administration of G-CSF in acute myocardial infarction: systematic review and meta-analysis. Heart 2008;94:610-6.
268. Haghighat A, Weiss D, Whalin MK, Cowan DP, Taylor WR. Granulocyte colony-stimulating factor and granulocyte macrophage colony-stimulating factor exacerbate atherosclerosis in apolipoprotein E-deficient mice. Circulation 2007;115:2049-54.
269. Menasche P, Hagege AA, Vilquin JT, Desnos M, Abergel E, Pouzet B, Bel A, Sarateanu S, Scorsin M, Schwartz K, Bruneval P, Benbunan M, Marolleau JP, Duboc D. Autologous skeletal myoblast transplantation for severe postinfarction left ventricular dysfunction. J Am Coll Cardiol 2003;41:1078-83.
270. Menasche P, Alfieri O, Janssens S, McKenna W, Reichenspurner H, Trinquart L, Vilquin JT, Marolleau JP, Seymour B, Larghero J, Lake S, Chatellier G, Solomon S, Desnos M, Hagege AA. The Myoblast Autologous Grafting in Ischemic Cardiomyopathy (MAGIC) trial: first randomized placebo-controlled study of myoblast transplantation. Circulation 2008;117:1189-200.
271. Kuhlmann MT, Kirchhof P, Klocke R, Hasib L, Stypmann J, Fabritz L, Stelljes M, Tian W, Zwiener M, Mueller M, Kienast J, Breithardt G, Nikol S. G-CSF/SCF reduces inducible arrhythmias in the infarcted heart potentially via increased connexin43 expression and arteriogenesis. J Exp Med 2006;203:87-97.
272. Overgaard M, Ripa RS, Wang Y, Jørgensen E, Kastrup J. Timing of granulocyte-colony stimulating factor treatment after acute myocardial infarction and recovery of left ventricular function: Results from the STEMMI trial. Int J Cardiol 2010;140:351-5.
273. Engelmann MG, Theiss HD, Theiss C, Huber A, Wintersperger BJ, Werle-Ruedinger AE, Schoenberg SO, Steinbeck G, Franz WM. G-CSF in patients suffering from late revascularized ST elevation myocardial infarction: analysis on the timing of G-CSF administration. Exp Hematol 2008;36:703-9.
274. Meluzin J, Mayer J, Groch L, Janousek S, Hornacek I, Hlinomaz O, Kala P, Panovsky R, Prasek J, Kaminek M, Stanicek J, Klabusay M, Koristek Z, Navratil M, Dusek L, Vinklarkova J. Autologous transplantation of mononuclear bone marrow cells in patients with acute myocardial infarction: the effect of the dose of transplanted cells on myocardial function. Am Heart J 2006;152:975-15.
275. Iwase T, Nagaya N, Fujii T, Itoh T, Murakami S, Matsumoto T, Kangawa K, Kitamura S. Comparison of angiogenic potency between mesenchymal stem cells and mononuclear cells in a rat model of hindlimb ischemia. Cardiovasc Res 2005;66:543-51.
276. Pitchford SC, Furze RC, Jones CP, Wengner AM, Rankin SM. Differential mobilization of subsets of progenitor cells from the bone marrow. Cell Stem Cell 2009;4:62-72.
277. Brouard N, Driessen R, Short B, Simmons PJ. G-CSF increases mesenchymal precursor cell numbers in the bone marrow via an indirect mechanism involving osteoclast-mediated bone resorption. Stem Cell Res 2010;5:65-75.
278. Kawada H, Fujita J, Kinjo K, Matsuzaki Y, Tsuma M, Miyatake H, Muguruma Y, Tsuboi K, Itabashi Y, Ikeda Y, Ogawa S, Okano H, Hotta T, Ando K, Fukuda K. Nonhematopoietic mesenchymal stem cells can be mobilized and differentiate into cardiomyocytes after myocardial infarction. Blood 2004;104:3581-7.
279. Delgaudine M, Lambermont B, Lancellotti P, Roelants V, Walrand S, Vanoverschelde JL, Pierard L, Gothot A, Beguin Y. Effects of granulocyte-colony-stimulating factor on progenitor cell mobilization and heart perfusion and function in normal mice. Cytotherapy 2011;13:237-47.
280. Cesselli D, Beltrami AP, Rigo S, Bergamin N, D'Aurizio F, Verardo R, Piazza S, Klaric E, Fanin R, Toffoletto B, Marzinotto S, Mariuzzi L, Finato N, Pandolfi M, Leri A, Schneider C, Beltrami CA, Anversa P. Multipotent progenitor cells are present in human peripheral blood. Circ Res 2009;104:1225-34.
281. Tatsumi K, Otani H, Sato D, Enoki C, Iwasaka T, Imamura H, Taniuchi S, Kaneko K, Adachi Y, Ikehara S. Granulocyte-colony stimulating factor increases donor mesenchymal stem cells in bone marrow and their mobilization into peripheral circulation but does not repair dystrophic heart after bone marrow transplantation. Circ J 2008;72:1351-8.
282. Delgaudine M, Gothot A, Beguin Y. Spontaneous and granulocyte-colony-stimulating factor-enhanced marrow response and progenitor cell mobilization in mice after myocardial infarction. Cytotherapy 2010;12:909-18.
283. Ince H, Petzsch M, Kleine HD, Eckard H, Rehders T, Burska D, Kische S, Freund M, Nienaber CA. Prevention of left ventricular remodeling with granulocyte colony-stimulating factor after acute myocardial infarction: final 1-year results of the Front-Integrated Revascularization and Stem Cell Liberation in Evolving Acute Myocardial Infarction by Granulocyte Colony-Stimulating Factor (FIRSTLINE-AMI) Trial. Circulation 2005;112:I73-I80.
284. Neumann FJ, Blasini R, Schmitt C, Alt E, Dirschinger J, Gawaz M, Kastrati A, Schomig A. Effect of glycoprotein IIb/IIIa receptor blockade on recovery of coronary flow and left ventricu-

lar function after the placement of coronary-artery stents in acute myocardial infarction. Circulation 1998;98:2695-701.
285. Ofili EO, Kern MJ, St Vrain JA, Donohue TJ, Bach R, al-Joundi B, Aguirre FV, Castello R, Labovitz AJ. Differential characterization of blood flow, velocity, and vascular resistance between proximal and distal normal epicardial human coronary arteries: analysis by intracoronary Doppler spectral flow velocity. Am Heart J 1995;130:37-46.
286. Vulliet PR, Greeley M, Halloran SM, MacDonald KA, Kittleson MD. Intra-coronary arterial injection of mesenchymal stromal cells and microinfarction in dogs. Lancet 2004;363:783-4.
287. Kang HJ, Lee HY, Na SH, Chang SA, Park KW, Kim HK, Kim SY, Chang HJ, Lee W, Kang WJ, Koo BK, Kim YJ, Lee DS, Sohn DW, Han KS, Oh BH, Park YB, Kim HS. 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:I145-I151.
288. Lutz M, Rosenberg M, Kiessling F, Eckstein V, Heger T, Krebs J, Ho AD, Katus HA, Frey N. Local injection of stem cell factor (SCF) improves myocardial homing of systemically delivered c-kit + bone marrow-derived stem cells. Cardiovasc Res 2008;77:143-50.
289. Theiss HD, Brenner C, Engelmann MG, Zaruba MM, Huber B, Henschel V, Mansmann U, Wintersperger B, Reiser M, Steinbeck G, Franz WM. Safety and efficacy of SITAgliptin plus GRanulocyte-colony-stimulating factor in patients suffering from Acute Myocardial Infarction (SITAGRAMI-Trial)--rationale, design and first interim analysis. Int J Cardiol 2010;145:282-4.
290. Baks T, van Geuns RJ, Biagini E, Wielopolski P, Mollet NR, Cademartiri F, Boersma E, van der Giessen WJ, Krestin GP, Duncker DJ, Serruys PW, de Feyter PJ. Recovery of left ventricular function after primary angioplasty for acute myocardial infarction. Eur Heart J 2005;26:1070-7.
291. Brodie BR, Stuckey TD, Kissling G, Hansen CJ, Weintraub RA, Kelly TA. Importance of infarct-related artery patency for recovery of left ventricular function and late survival after primary angioplasty for acute myocardial infarction. J Am Coll Cardiol 1996;28:319-25.
292. Stone GW, Grines CL, Cox DA, Garcia E, Tcheng JE, Griffin JJ, Guagliumi G, Stuckey T, Turco M, Carroll JD, Rutherford BD, Lansky AJ. Comparison of angioplasty with stenting, with or without abciximab, in acute myocardial infarction. N Engl J Med 2002;346:957-66.
293. Jørgensen E, Madsen T, Kastrup J. Comparison of the left ventricular electromechanical map before percutaneous coronary stent revascularization and at one-month follow-up in patients with a recent ST elevation infarction. Catheter Cardiovasc Interv 2005;64:153-9.
294. Schroeder AP, Houlind K, Pedersen EM, Nielsen TT, Egeblad H. Serial magnetic resonance imaging of global and regional left ventricular remodeling during 1 year after acute myocardial infarction. Cardiology 2001;96:106-14.
295. Petersen SE, Voigtlander T, Kreitner KF, Horstick G, Ziegler S, Wittlinger T, Abegunewardene N, Schmitt M, Schreiber WG, Kalden P, Mohrs OK, Lippold R, Thelen M, Meyer J. Late improvement of regional wall motion after the subacute phase of myocardial infarction treated by acute PTCA in a 6-month follow-up. J Cardiovasc Magn Reson 2003;5:487-95.
296. Nijveldt R, Beek AM, Hirsch A, Stoel MG, Hofman MB, Umans VA, Algra PR, Twisk JW, van Rossum AC. Functional recovery after acute myocardial infarction: comparison between angiography, electrocardiography, and cardiovascular magnetic resonance measures of microvascular injury. J Am Coll Cardiol 2008;52:181-9.
297. Dill T, Schachinger V, Rolf A, Mollmann S, Thiele H, Tillmanns H, Assmus B, Dimmeler S, Zeiher AM, Hamm C. Intracoronary administration of bone marrow-derived progenitor cells improves left ventricular function in patients at risk for adverse remodeling after acute ST-segment elevation myocardial infarction: results of the Reinfusion of Enriched Progenitor cells And Infarct Remodeling in Acute Myocardial Infarction study (REPAIR-AMI) cardiac magnetic resonance imaging substudy. Am Heart J 2009;157:541-7.
298. Gerber BL, Wijns W, Vanoverschelde JL, Heyndrickx GR, De BB, Bartunek J, Melin JA. Myocardial perfusion and oxygen consumption in reperfused noninfarcted dysfunctional myocardium after unstable angina: direct evidence for myocardial stunning in humans. J Am Coll Cardiol 1999;34:1939-46.
299. Hombach V, Grebe O, Merkle N, Waldenmaier S, Hoher M, Kochs M, Wohrle J, Kestler HA. Sequelae of acute myocardial infarction regarding cardiac structure and function and their prognostic significance as assessed by magnetic resonance imaging. Eur Heart J 2005;26:549-57.
300. Fieno DS, Hillenbrand HB, Rehwald WG, Harris KR, Decker RS, Parker MA, Klocke FJ, Kim RJ, Judd RM. Infarct resorption, compensatory hypertrophy, and differing patterns of ventricular remodeling following myocardial infarctions of varying size. J Am Coll Cardiol 2004;43:2124-31.
301. Richard V, Murry CE, Reimer KA. Healing of myocardial infarcts in dogs. Effects of late reperfusion. Circulation 1995;92:1891-901.
302. Rogers WJ, Jr., Kramer CM, Geskin G, Hu YL, Theobald TM, Vido DA, Petruolo S, Reichek N. Early contrast-enhanced MRI predicts late functional recovery after reperfused myocardial infarction. Circulation 1999;99:744-50.
303. Saeed M, Lund G, Wendland MF, Bremerich J, Weinmann H, Higgins CB. Magnetic resonance characterization of the peri-infarction zone of reperfused myocardial infarction with necrosis-specific and extracellular nonspecific contrast media. Circulation 2001;103:871-6.
304. Oshinski JN, Yang Z, Jones JR, Mata JF, French BA. Imaging time after Gd-DTPA injection is critical in using delayed enhancement to determine infarct size accurately with magnetic resonance imaging. Circulation 2001;104:2838-42.
305. Kim RJ, Fieno DS, Parrish TB, Harris K, Chen EL, Simonetti O, Bundy J, Finn JP, Klocke FJ, Judd RM. Relationship of MRI delayed contrast enhancement to irreversible injury, infarct age, and contractile function. Circulation 1999;100:1992-2002.
306. Fieno DS, Kim RJ, Chen EL, Lomasney JW, Klocke FJ, Judd RM. Contrast-enhanced magnetic resonance imaging of myocardium at risk: distinction between reversible and irreversible injury throughout infarct healing. J Am Coll Cardiol 2000;36:1985-91.
307. Lipinski MJ, Biondi-Zoccai GG, Abbate A, Khianey R, Sheiban I, Bartunek J, Vanderheyden M, Kim HS, Kang HJ, Strauer BE, Vetrovec GW. Impact of intracoronary cell therapy on left ventricular function in the setting of acute myocardial infarction: a collaborative systematic review and meta-analysis of controlled clinical trials. J Am Coll Cardiol 2007;50:1761-7.

308. Kawamoto A, Iwasaki H, Kusano K, Murayama T, Oyamada A, Silver M, Hulbert C, Gavin M, Hanley A, Ma H, Kearney M, Zak V, Asahara T, Losordo DW. CD34-positive cells exhibit increased potency and safety for therapeutic neovascularization after myocardial infarction compared with total mononuclear cells. Circulation 2006;114:2163-9.
309. Pittenger MF, Mackay AM, Beck SC, Jaiswal RK, Douglas R, Mosca JD, Moorman MA, Simonetti DW, Craig S, Marshak DR. Multilineage potential of adult human mesenchymal stem cells. Science 1999;284:143-7.
310. Haack-Sørensen M, Friis T, Bindslev L, Mortensen S, Johnsen HE, Kastrup J. Comparison of different culture conditions for human mesenchymal stromal cells for clinical stem cell therapy. Scand J Clin Lab Invest 2008;68:192-203.
311. Haack-Sørensen M, Friis T, Kastrup J. Mesenchymal stromal cell and mononuclear cell therapy in heart disease. Future Cardiology 2008;4:481-94.
312. Hare JM, Traverse JH, Henry TD, Dib N, Strumpf RK, Schulman SP, Gerstenblith G, DeMaria AN, Denktas AE, Gammon RS, Hermiller JB, Jr., Reisman MA, Schaer GL, Sherman W. A randomized, double-blind, placebo-controlled, dose-escalation study of intravenous adult human mesenchymal stem cells (prochymal) after acute myocardial infarction. J Am Coll Cardiol 2009;54:2277-86.
313. Hofmann M, Wollert KC, Meyer GP, Menke A, Arseniev L, Hertenstein B, Ganser A, Knapp WH, Drexler H. Monitoring of bone marrow cell homing into the infarcted human myocardium. Circulation 2005;111:2198-202.
314. He G, Zhang H, Wei H, Wang Y, Zhang X, Tang Y, Wei Y, Hu S. In vivo imaging of bone marrow mesenchymal stem cells transplanted into myocardium using magnetic resonance imaging: a novel method to trace the transplanted cells. Int J Cardiol 2007;114:4-10.
315. Kjær A, Lebech AM. [Leukocyte scintigraphy: indications and diagnostic value]. Ugeskr Laeger 2001;163:4380-4.
316. Jin Y, Kong H, Stodilka RZ, Wells RG, Zabel P, Merrifield PA, Sykes J, Prato FS. Determining the minimum number of detectable cardiac-transplanted 111In-tropolone-labelled bone-marrow-derived mesenchymal stem cells by SPECT. Phys Med Biol 2005;50:4445-55.
317. Gholamrezanezhad A, Mirpour S, Ardekani JM, Bagheri M, Alimoghadam K, Yarmand S, Malekzadeh R. Cytotoxicity of 111In-oxine on mesenchymal stem cells: a time-dependent adverse effect. Nucl Med Commun 2009;30:210-6.
318. Bindslev L, Haack-Sørensen M, Bisgaard K, Kragh L, Mortensen S, Hesse B, Kjaer A, Kastrup J. Labelling of human mesenchymal stem cells with indium-111 for SPECT imaging: effect on cell proliferation and differentiation. Eur J Nucl Med Mol Imaging 2006;33:1171-7.
319. Schächinger V, Aicher A, Dobert N, Rover R, Diener J, Fichtlscherer S, Assmus B, Seeger FH, Menzel C, Brenner W, Dimmeler S, Zeiher AM. Pilot trial on determinants of progenitor cell recruitment to the infarcted human myocardium. Circulation 2008;118:1425-32.
320. Goussetis E, Manginas A, Koutelou M, Peristeri I, Theodosaki M, Kollaros N, Leontiadis E, Theodorakos A, Paterakis G, Karatasakis G, Cokkinos DV, Graphakos S. Intracoronary infusion of CD133+ and CD133-CD34+ selected autologous bone marrow progenitor cells in patients with chronic ischemic cardiomyopathy: cell isolation, adherence to the infarcted area, and body distribution. Stem Cells 2006;24:2279-83.
321. Caveliers V, De KG, Everaert H, Van R, I, Van CG, Verheye S, Roland J, Schoors D, Franken PR, Schots R. In vivo visualization of 111In labeled CD133+ peripheral blood stem cells after intracoronary administration in patients with chronic ischemic heart disease. Q J Nucl Med Mol Imaging 2007;51:61-6.
322. Kurpisz M, Czepczynski R, Grygielska B, Majewski M, Fiszer D, Jerzykowska O, Sowinski J, Siminiak T. Bone marrow stem cell imaging after intracoronary administration. Int J Cardiol 2007;121:194-5.
323. Zhou R, Thomas DH, Qiao H, Bal HS, Choi SR, Alavi A, Ferrari VA, Kung HF, Acton PD. In vivo detection of stem cells grafted in infarcted rat myocardium. J Nucl Med 2005;46:816-22.
324. Tossios P, Krausgrill B, Schmidt M, Fischer T, Halbach M, Fries JW, Fahnenstich S, Frommolt P, Heppelmann I, Schmidt A, Schomacker K, Fischer JH, Bloch W, Mehlhorn U, Schwinger RH, Muller-Ehmsen J. Role of balloon occlusion for mononuclear bone marrow cell deposition after intracoronary injection in pigs with reperfused myocardial infarction. Eur Heart J 2008;29:1911-21.
325. Aicher A, Brenner W, Zuhayra M, Badorff C, Massoudi S, Assmus B, Eckey T, Henze E, Zeiher AM, Dimmeler S. Assessment of the tissue distribution of transplanted human endothelial progenitor cells by radioactive labeling. Circulation 2003;107:2134-9.
326. Brenner W, Aicher A, Eckey T, Massoudi S, Zuhayra M, Koehl U, Heeschen C, Kampen WU, Zeiher AM, Dimmeler S, Henze E. 111In-labeled CD34+ hematopoietic progenitor cells in a rat myocardial infarction model. J Nucl Med 2004;45:512-8.
327. Hou D, Youssef EA, Brinton TJ, Zhang P, Rogers P, Price ET, Yeung AC, Johnstone BH, Yock PG, March KL. Radiolabeled cell distribution after intramyocardial, intracoronary, and interstitial retrograde coronary venous delivery: implications for current clinical trials. Circulation 2005;112:I150-I156.
328. Amsalem Y, Mardor Y, Feinberg MS, Landa N, Miller L, Daniels D, Ocherashvilli A, Holbova R, Yosef O, Barbash IM, Leor J. Iron-oxide labeling and outcome of transplanted mesenchymal stem cells in the infarcted myocardium. Circulation 2007;116:I38-I45.
329. Terrovitis J, Stuber M, Youssef A, Preece S, Leppo M, Kizana E, Schar M, Gerstenblith G, Weiss RG, Marban E, Abraham MR. Magnetic resonance imaging overestimates ferumoxide-labeled stem cell survival after transplantation in the heart. Circulation 2008;117:1555-62.
330. Gyongyosi M, Blanco J, Marian T, Tron L, Petnehazy O, Petrasi Z, Hemetsberger R, Rodriguez J, Font G, Pavo IJ, Kertesz I, Balkay L, Pavo N, Posa A, Emri M, Galuska L, Kraitchman DL, Wojta J, Huber K, Glogar D. Serial Noninvasive In Vivo Positron Emission Tomographic Tracking of Percutaneously Intramyocardially Injected Autologous Porcine Mesenchymal Stem Cells Modified for Transgene Reporter Gene Expression. Circ Cardiovasc Imaging 2008;1:94-103.
331. Zimmermann WH, Melnychenko I, Wasmeier G, Didie M, Naito H, Nixdorff U, Hess A, Budinsky L, Brune K, Michaelis B, Dhein S, Schwoerer A, Ehmke H, Eschenhagen T. Engineered heart tissue grafts improve systolic and diastolic function in infarcted rat hearts. Nat Med 2006;12:452-8.
332. Feinberg AW, Feigel A, Shevkoplyas SS, Sheehy S, Whitesides GM, Parker KK. Muscular thin films for building actuators and powering devices. Science 2007;317:1366-70.