Published in final edited form as:

Microvasc Res. 2007 ; 74(2-3): 159–171.

Therapeutic Myocardial Angiogenesis

Marie-Ange Renault and Douglas W. Losordo

Northwestern University Feinberg School of Medicine Feinberg Cardiovascular Research Institute

Therapeutic Myocardial Angiogenesis

Armed with an improved understanding of the mediators of angiogenesis, physicians and scientists have made significant efforts at harnessing this naturally occurring process in order to treat patients with a variety of peripheral vascular and coronary ischemic syndromes. There is a growing population of patients with end-stage coronary artery disease (CAD) who are no longer candidates for mechanical revascularization, yet suffer from chronic myocardial ischemia who may benefit from regeneration of the depleted microvasculature.

A/

Ischemic muscle has certain features that should provide a reasonable substrate for angiogenic therapy in that hypoxia stimulates the expression of angiogenic growth factor receptors (Goldberg and Schneider 1994; Brogi et al. 1996; Levy et al. 1996; Waltenberger et al. 1996; Asahara et al. 1997). There are numerous methods of angiogenic cytokine delivery. One method involves delivering the cytokine in a recombinant protein form. Since the protein is the active agent, it is reasonable to consider the use of recombinant protein as the most conventional and practical means for delivery of angiogenic cytokines; however, there are several important potential limitations to recombinant protein therapy. First, a single dose of protein may need to be relatively high to overcome a short tissue half-life, unless a mechanism for prolonged retention can be incorporated into the delivery technique (Sellke et al. 1998). Secondly, recombinant proteins administered in high doses may result in significant side effects, such as hypotension. (Cuevas et al. 1991; Hariawala et al. 1996) Third, recombinant proteins can be difficult and costly to produce.

2. Gene therapy—Angiogenic cytokines can also be delivered via gene therapy that allows for transcription and translation of angiogenic protein locally within the targeted tissue. Genes can be transferred using non-viral and viral methods. Skeletal and cardiac muscle have been shown to be feasible targets for gene transfer (Wolff et al. 1990; Gal et al. 1993) and express genes encoded in plasmids as well as transgenes incorporated into viral vectors. Further, efficiency of gene transfection is enhanced significantly in ischemic tissue (Takeshita et al. 1996; Tsurumi et al. 1996). Non-viral methods generally involve delivery of naked plasmid encoding the gene of interest or delivery of DNA material in a liposomal vehicle. Because of circulating nucleases, delivery of DNA is limited by shorter half-life in targeted tissue, and therefore, the time for gene expression may be limited. However, delivery of non-virally encoded DNA may allow for more meaningful biological effects since protein is secreted locally resulting in a “bystander” effect. *In vitro* and *in vivo* studies have also shown that despite

Address for manuscript correspondence: Douglas W. Losordo, M.D. Director, Feinberg Cardiovascular Research Institute Northwestern University Feinberg School of Medicine 303 E Chicago Ave. Tarry 12-703 Chicago, IL 60611 Tel: 312-695-0072 Fax: 312-695-0047 E-mail: d-losordo@northwestern.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

lower gene transfer efficiency with non-viral methods, site-specific administration of the gene still results in a meaningful biological effect at the local tissue (Takeshita et al. 1994) (Losordo et al. 1994). An additional advantage of non-viral gene transfer is that it allows for repeat administration of DNA since an immune response, expected after exposure to viral vectors (generally adenovirus or retrovirus), does not occur following transfer of naked DNA.

3. Cell therapy—The recent identification of adult and embryonic progenitor/stem cells as well as the discovery that vasculogenesis occurs in the adult ischemic tissue (Asahara et al. 1997), has triggered attempts to use cell therapy to promotes revascularization of ischemic tissues.

Endothelial Progenitor cells (EPCs): Initially, Flk-1 and a second marker, CD34, shared by angioblasts and hematopoietic cells were used to isolate putative angioblasts from the mononuclear cell fraction of peripheral blood (Asahara et al. 1997). Meanwhile, endothelial progenitor cells (EPCs) subsequently were isolated from human umbilical cord blood, bone marrow (BM)-derived mononuclear cells, and CD34⁺ or CD133⁺ hematopoietic SCs (Shi et al. 1998; Asahara et al. 1999; Murohara et al. 2000; Rafii 2000). These cells differentiate into endothelial cells, as shown by expression of various endothelial proteins (KDR, von Willebrand factor, endothelial nitric oxidase synthase, VE-cadherin, CD146), uptake of DiI-acetylated low-density lipoprotein and binding of lectin. Embryonic EPCs (expressing Tie2 and thrombomodulin) have also been used (Kupatt et al. 2005).

Mesenchymal Stem Cells (MSCs): Another source of adult stem cells being explored for possible cardiac regeneration is mesenchymal stem cells (or marrow stromal cells, MSCs). The stem cells for nonhematopoietic tissues are currently referred to MSCs because of their ability to differentiate into the cells of mesenchymal origin such as osteoblasts, chondrocytes, astrocytes, and skeletal muscles. The MSCs can be isolated from BM or adipose tissue (Moon et al. 2006; Ohnishi et al. 2007), expanded in culture and demonstrate multi-lineage differentiation capacity (Prockop 1997; Pittenger et al. 1999). The advantage of this cell population is the ease of acquisition and expansion in culture. This extensive capacity for clinical scale expansion in vitro has facilitated the development of preclinical models as well as clinical studies designed to assess the safety, feasibility and efficacy of MSC transplantation in a variety of diseases.

4. Mobilization of BM-derived stem/progenitor cells—Recently, there has been a growing body of evidence that endogenous mobilization of BM cells could be obtained by local or systemic use of vascular endothelial growth factor (VEGF) (Asahara et al. 1999; Kalka et al. 2000; Rafii et al. 2002), placental growth factor (PlGF) (Luttun et al. 2002), stromal cell derived factor-1 (SDF-1) (Yamaguchi et al. 2003), granulocyte colony stimulating factor (G-CSF) (Orlic et al. 2001) or granulocyte-monocyte colony stimulating factor (GM-CSF) (Takahashi et al. 1999; Seiler et al. 2001). Various stem/progenitor cell populations within BM can exert their therapeutic effect by direct cellular differentiation of stem cells as well as from their capacity to release angiogenic factors such as VEGF-A, VEGF-C (Wartiovaara et al. 1998), angiopoietins (Huang et al. 2000) and matrix metalloproteinases (Coussens et al. 2000). Systemic injection of G-CSF and stem cell factor (SCF) (Orlic et al. 2001) in a mouse model of acute myocardial infarction (MI) decreased mortality by 68%, infarct size by 40%, cavity dilation by 26%, and diastolic stress by 70%. Ejection fraction progressively increased and hemodynamics significantly improved. Under this basis, the strategy of endogenous mobilization has emerged as a viable therapeutic option for ischemic cardiovascular diseases. In particular, patients with acute myocardial infarction or chronic severe ischemic diseases who can not tolerate the invasive procedures necessary for the harvest or transplantation of stem cells may be ideal candidates for this therapeutic strategy.

B/ Clinical Trials

1. Clinical Trials using protein/gene therapy (table 1)

Clinical Trials using VEGF A Protein/gene therapy: Therapy with VEGF protein has been shown in pre-clinical studies to enhance collateral blood flow in an animal model of chronic myocardial ischemia (Banai et al. 1994; Pearlman et al. 1995; Harada et al. 1996; Hariawala et al. 1996; Lopez et al. 1998). Several Phase I studies have been performed to assess the safety and feasibility of recombinant VEGF protein for therapeutic angiogenesis in patients (Table 1). The potential of recombinant VEGF for therapeutic angiogenesis for coronary artery disease was further tested in the Phase II VIVA Trial (Henry et al. 2003). This was a double-blind placebo-controlled trial where 178 patients with inoperable coronary artery disease and stable angina were randomized to receive placebo or one of two doses of recombinant VEGF: 17 ng/kg/min (low-dose) and 50 ng/kg/min (high-dose). The VIVA trial showed recombinant VEGF delivered by the intracoronary and intravenous routes to be safe (no major hypotension, no thrombocytopenia, proteinuria, or retinopathy, no development of cancer), and well-tolerated; however, the study failed to show a significant benefit in exercise time in patients treated with recombinant VEGF at 60 days after treatment. Possible explanations for the findings of this study include an inadequate dose of recombinant VEGF, suboptimal route of administration, and inappropriate selection of time points to assess efficacy. This study also demonstrated the potential importance of the placebo effect in uncontrolled trials of therapeutic angiogenesis.

Concurrent with studies of recombinant VEGF was clinical investigation evaluating the delivery of naked VEGF DNA for the treatment of end-stage myocardial ischemia. Early studies in humans involved direct delivery of plasmid encoding VEGF₁₆₅ (phVEGF₁₆₅) during thoracotomy (Losordo et al. 1998; Symes et al. 1999; Vale et al. 2000). These phase I studies showed that plasma VEGF levels is increased after treatment providing proof of concept that direct gene transfection can result in gene expression, and all patients experience improvement of symptoms and/or objective evidence of improvement in myocardial perfusion scanning. Of course, the major limitations of delivery of angiogenic factors via thoracotomy are the inability to have an effective control group in clinical trials as well as the need for surgery and the attendant risks of surgery. An endovascular approach to gene transfer was shown to be feasible in swine studies using NOGA electromechanical mapping (Vale et al. 1999). The first study of NOGA directed myocardial gene therapy in humans was reported in 2001 (Vale et al. 2001).

The recently reported Euroinject One study was a trial of 80 patients with CCS Class III or IV angina and no-option for conventional revascularization randomized to receive direct myocardial injection of phVEGF₁₆₅ or placebo using NOGA guidance (Kastrup et al. 2005). The primary endpoint of this study was change in perfusion defect scores from baseline at three months. In the phVEGF₁₆₅ group, there was a significant improvement in stress-induced perfusion defects at three months compared to baseline while a similar improvement was not seen in the control group. The difference between the two groups, however, was not significant. There were also significant improvements in wall motion as assessed by NOGA and contrast ventriculography in the phVEGF₁₆₅ group compared to the control group. Overall, there were no adverse events due to gene therapy in this study, though there were five adverse events related to NOGA mapping.

Several early preclinical studies performed in pigs and dogs showed feasibility of myocardial gene transfer using adenovirus encoding VEGF (Mack et al. 1998; Lazarous et al. 1999; Lee et al. 2000). The first human experience with AdVEGF₁₂₁ involving 21 patients did not demonstrated any adverse events because of direct myocardial delivery of AdVEGF₁₂₁. The REVASC study is the largest clinical trial to date evaluating the efficacy of AdVEGF₁₂₁ for myocardial ischemia (Stewart 2002). This study was a Phase II randomized multi-center trial

that enrolled 67 patients to receive AdVEGF₁₂₁ delivered by direct intramyocardial injections via minithoracotomy or to continue with optimization of medical therapy. The primary endpoint, time to ischemia on treadmill testing, was significantly improved in the AdVEGF₁₂₁ treated patients compared to the medical therapy patients ($P=0.024$). There were also significant improvements in the Seattle Angina Questionnaire in the AdVEGF₁₂₁ treated patients compared to the medically treated patients. Since this was not a blinded study (patients randomized to treatment all underwent thoracotomy) the authors could not exclude the placebo effect as the sole cause for improvement in AdVEGF₁₂₁ treated patients. There were also four patients in the AdVEGF₁₂₁ group who suffered complications from thoracotomy highlighting a significant limitation of the surgical approach to gene therapy.

Clinical Trials using VEGF-2 gene therapy: A first pilot study enrolled six patients with chronic symptomatic myocardial ischemia and no option for conventional mechanical revascularization who were randomized to receive 200 µg of plasmid encoding VEGF-2 (phVEGF-2) or placebo by NOGA directed injection (Vale et al. 2001). Patients treated with phVEGF-2 presented improved angina with no adverse events. This study confirmed the safety and feasibility of endovascular delivery of gene therapy in humans. As a follow-up of this Phase I pilot study, a Phase I/II randomized, double-blind, placebo-controlled trial of VEGF-2 therapy was performed (Losordo et al. 2002). A total of 27 patients were planned for randomization into one of three treatment groups: 200µg, 800µg, or 2000µg of phVEGF-2. In each dose cohort, patients were randomized to receive the specified dose of phVEGF-2 or placebo in a 2:1 ratio. At 12 weeks after treatment, there was a statistically significant improvement in Canadian Cardiovascular Society (CCS) angina class in phVEGF-2 treated versus placebo-treated patients (-1.3 versus -0.1 , $P=0.04$). There was a trend toward improvement in change in exercise duration (91.8 seconds versus 3.9 seconds), functional improvement by $> \text{or} = 2$ CCS classes (9 of 12 versus 1 of 6), and Seattle Angina Questionnaire data in phVEGF-2 patients compared to placebo treated patients. Importantly, no adverse events were noted either as a result of NOGA-guided gene therapy delivery or the gene therapy itself. Only one patient was treated in the third dose cohort and received placebo treatment. A phase IIb pivotal trial of endovascular delivery of phVEGF-2 in patients with chronic myocardial ischemia using the Stiletto™ (Boston Scientific, Natick, MA) endocardial injection catheter was recently completed and results are pending.

Clinical Trials using FGF2 (bFGF) Protein: Animal models have shown that locally administered FGF protein can induce angiogenesis (Unger et al. 1994; Lazarous et al. 1995). Phase I studies have been performed evaluating the safety and feasibility of recombinant FGF2 for therapeutic angiogenesis in patients with chronic myocardial ischemia (Schumacher et al. 1998; Sellke et al. 1998; Laham et al. 1999; Stegmann et al. 2000; Stegmann et al. 2000; Udelson et al. 2000; Unger et al. 2000). The FIRST trial which is the largest study to date of recombinant FGF2 for the treatment of chronic myocardial ischemia (Simons et al. 2002) confirmed that administration of recombinant FGF is safe and feasible, but revealed that the efficacy of recombinant FGF as an agent for therapeutic angiogenesis was questionable. It is possible that the recombinant protein form of recombinant FGF has too short of a half-life when administered via an intravascular route to provide for effective angiogenesis. Another limitation to this study was the relatively high baseline exercise times in the patient population (540 seconds). This suggests that while these patients were no-option for revascularization, they may have not been symptom-limited enough to provide a reliable substrate for evaluation of therapeutic angiogenesis.

Clinical Trials of FGF5 gene therapy: Giordano, et al. used a swine model of chronic myocardial ischemia to show the feasibility of gene transfer of human FGF5 delivered by a single intracoronary infusion with an adenovirus vector (Ad5-FGF5) (Giordano et al. 1996). This method of gene transfer resulted in a sustained in situ production of FGF messenger RNA

and protein for 12 weeks after treatment and enhanced collateral formation, collateral blood flow, and relief of stress-induced ischemia. The first clinical trial enrolling 79 patients with CCS Class II or III angina (Grines et al. 2002) showed no major adverse events that resulted from intracoronary delivery of Ad5-FGF5. The efficacy of Ad5-FGF5 therapy was further examined in the AGENT 2 and AGENT 3 studies. The AGENT 2 study was a further dose finding study while the AGENT 3 was designed as the pivotal efficacy study of Ad5-FGF5 for therapeutic angiogenesis for myocardial ischemia (presented at Transcatheter Therapeutics Scientific Sessions, 2004). Patients were included in this study with stable exertional angina despite optimal medical therapy, anatomy suitable for but not *immediately* requiring revascularization, and ability to exercise on a treadmill test for at least 3 minutes. A total of 450 patients were planned to be randomized to receive placebo, 1×10^9 virus particles, or 1×10^{10} virus particles as an intracoronary injection. The primary endpoint of the study was change in treadmill exercise duration (high-dose vs. placebo) at 12 weeks.

The trial was stopped early by the Data Safety Monitoring Board after 300 patients were enrolled in the study due to fact that an early analysis of the data showed that the trial could not show superiority of the angiogenesis strategy over placebo. There were no safety concerns. Analysis of available data from the study showed that there were improvements in exercise duration in all groups but improvement was not significant between groups. Further, exercise duration continued to improve at 12 weeks after therapy. Interestingly, subgroup analysis indicated that patients greater than 55 years of age and with Class 3 or 4 angina showed evidence of significant benefit, reinforcing the importance of trial design in evaluation of therapeutic angiogenesis.

2. Cell Therapy for Angiogenesis for CAD (Table 2)—In animal models of ischemia, the systemic administration of ex-vivo expanded EPCs (Kawamoto et al. 2001), direct transplantation of purified human CD34+ cells (Kawamoto et al. 2001; Kocher et al. 2001; Botta et al. 2004) following infarction improved neovascularization and myocardial function. Cells have been shown to incorporate into foci of myocardial neovascularization, Total mononuclear cell fraction of BM (Kamihata et al. 2001; Liu et al. 2004; Bhakta et al. 2006) or umbilical cord blood (Murohara et al. 2000) have also been used. The rationale of using unselected BM mononuclear cells is based on the premise that BM or peripheral blood cells include multiple cytokines related with neovascularization and stem/progenitor cell populations which promote vasculogenesis (Liu et al. 2004; Takahashi et al. 2006).

Cell Therapy using unselected BM-MNCs: Unselected BM-MNCs have been used in more than 15 clinical trials (Table 2). Cells have been harvested either directly from the BM or in the peripheral blood after G-CSF injection. All the studies showed that cell therapy is feasible and safe. The results obtained after the first phase I studies were promising. The first study reported by Strauer et al. (Strauer et al. 2002) showed that the cell therapy group experienced significant improvements in stroke volume index, left ventricular end-systolic volume (ESV) and contractility, and myocardial perfusion of the infarct region. The BOOST trial (Wollert et al. 2004), reported an improved LVEF and reduced ESV in treated patient supporting a favorable effect of BM-MNCs therapy on cardiac function after MI. Nevertheless the more recent studies involving greater numbers of patients tend to show that cell therapy using unselected BM-MNC does not have significant effects (Lunde et al. 2006; Meyer et al. 2006).

Tse et al. published their experience in eight patients with severe ischemic heart disease using NOGA mapping for intramyocardial delivery of bone marrow cells (BMC) (Tse et al. 2003). At three months, there were improvements in symptoms, myocardial perfusion, and function in the ischemia area on MRI. In a similar pilot study, ten patients underwent intramyocardial delivery of autologous BMC using NOGA guidance (Fuchs et al. 2001). Again, no adverse events were noted with treatment. At three months, there was a significant improvement in

CCS angina score compared to baseline (3.1 ± 0.3 vs. 2.0 ± 0.94 , $P = 0.001$) and stress induced ischemia scores (2.1 ± 0.8 vs. 1.6 ± 0.8 , $P < 0.001$). A non-significant increase in treadmill exercise times was also noted.

Clinical trial using selected EPC: Several clinical trials have used selected EPCs to treat patients with CAD. The TOPCARE-AMI study (Assmus et al. 2002), and then the REPAIR-MI (Schachinger et al. 2006) and REPAIR-CHF (Assmus et al. 2006) studies evaluated BM or peripheral blood derived progenitor cells, administered via intracoronary infusion for recent or chronic MI, respectively. The latter studies were randomized and placebo controlled, revealing statistically significant improvements in LV function in treated patients, providing important proof of concept data. In a study involving six patients undergoing CABG after myocardial infarction, autologous CD133+ bone marrow cells were directly injected into the infarct border zone (Stamm et al. 2003). Between three and nine months after surgery, four of the patients experience improvement in global left ventricular function and tissue perfusion improved on SPECT imaging. There were no adverse events related to autologous bone marrow cell therapy in this study. The second study using CD133+ cells (Bartunek et al. 2005) was a phase I/II clinical trial involving 35 patients with AMI, Patient from the cell treated group presented an improved LVEF and CS compared to the control group. Increased restenosis was observed.

Three early phase studies using CD34+ cells have been reported. In all 3 studies, the patients were treated with G-CSF followed by apheresis and selection of CD34+ cells. One study involved 5 patient with old MI (Archundia et al. 2005), the second involved 20 patient with AMI (Steinwender et al. 2006).

The most recent trial of CD34+ cell therapy included 24 patients with intractable angina who were not candidates for myocardial revascularization (Losordo 2007). This was a randomized, double-blind, placebo-controlled study involving transplantation of autologous CD34+ cells to ischemic myocardial zones identified by NOGA mapping. There were three dose-cohorts: 5×10^4 cells, 1×10^5 cells, 2.5×10^5 cells, and saline control. Eight patients were assigned to each dose cohort and received treatment or placebo in a 3:1 ratio. Safety analysis showed no evidence of adverse events attributable to the intramyocardial injection of cells. Careful monitoring for ventricular arrhythmias (patients were required to have an ICD or to wear a LifeVest wearable defibrillator) showed no incidence of significant ventricular arrhythmias. Efficacy analysis showed trends favoring CD34+ cells vs. placebo in most of the endpoints including reduction in angina frequency, nitroglycerine usage, CCS class, exercise time and Seattle Angina Questionnaire quality of life assessment. A phase IIb study is underway to continue to evaluate this therapeutic approach.

Clinical trial using selected MSCs: Several studies have reported that administration of MSCs in animal models of MI also improves cardiac fuction after MI (Nagaya et al. 2004; Olivares et al. 2004; Silva et al. 2005). One of the mechanisms by which MSCs promotes tissue repair after ischemia, is through the process of neovascularization mainly because MSCs produce angiogenic cytokines including VEGF (Kinnaird et al. 2004; Kinnaird et al. 2004; Ma et al. 2005; Tang et al. 2005; Zhang et al. 2005) and also because of their ability to differentiate into ECs and SMCs (Kinnaird et al. 2004; Kinnaird et al. 2004; Silva et al. 2005). MSCs have to been reported to be superior to angiogenic growth factor genes for improving myocardial performance in the mouse model of acute myocardial infarction (Shyu et al. 2006). To date two clinical trials have assessed the therapeutic potential of MSCs for MI. In both studies, MSCs have been selected by 7 day-culture. The first study by Chen (Chen et al. 2004) which involved 69 patients have shown that MSCs administration is safe and feasible. The MSC-treated patients presented improved LVEF, increase perfusion and decrease ESV, EDV compared to the control group. In the second study (Katrtsis et al. 2005), eleven patients with

an anteroseptal MI were treated with both MSCs and EPCs. Five of the 11 treated patients showed improved myocardial contractibility.

3. G-CSF therapy for CAD (Table 2)—In several clinical trials, G-CSF has been used to promote mobilization of progenitor/stem cells from the bone marrow. It has been used as a pretreatment before autologous cells transplantation or as a therapy by itself. The first study examining the feasibility and efficacy of G-CSF therapy was done by Kuethe in 2004 (Kuethe et al. 2004). G-CSF was injected subcutaneously at 10 µg/kg/day for 7 days. The peak of CD34⁺ circulating cells was reached at 5 days. After 3 months patients showed improved LVEF without any severe side effect but the MAGIC trial (Kang et al. 2004), reported soon after, revealed that patients treated with G-CSF presented an unexpectedly high rate of in-stent restenosis. This side effect has been shown to be avoided in 2 studies using lower dose of G-CSF (3–5 µg/kg/day) (Valgimigli et al. 2005; Suzuki et al. 2006). And the largest study to date using G-CSF therapy (10 µg/day/kg) and involving 114 patients did not demonstrate a significant increase of restenosis in the G-CSF group compared with the control (Zohlhofer et al. 2006). Nevertheless, stem cell mobilization by G-CSF therapy alone does not seem to have any influence on infarct size and left ventricular function (Ripa et al. 2006; Zohlhofer et al. 2006).

4. Combined gene and cell therapy—In animal models of MI, researchers have tried to combine gene and cell therapy but among five studies (Chachques et al. 2004; Azarnoush et al. 2005; Schuh et al. 2005; Yang et al. 2006), only 2 in which skeletal myoblasts transplantation was combined with HIF-1α gene therapy (Azarnoush et al. 2005) or CD34⁺ cells injection were combined with VEGF-2 gene therapy (Shintani et al. 2006) demonstrated enhanced therapeutic effects compared to cell or gene therapy alone.

Another strategy is to promote the proangiogenic activity of progenitor/stem cells by transfected cells, over expressing VEGF (Iwaguro et al. 2002; Matsumoto et al. 2005; Yau et al. 2005), angiopoietin1 (Chen et al. 2005) or stem cell factor (Askari et al. 2003). Researchers have also tried to promote the survival of progenitor cells by using cells over expressing IGF (Liu et al. 2004; Yau et al. 2005).

C/ Conclusion/perspective

1. Safety concerns regarding therapeutic angiogenesis—There are several theoretical safety concerns regarding the delivery of angiogenic cytokines in an effort to induce therapeutic angiogenesis for the treatment of ischemic cardiovascular disease. These concerns can be divided into acute effects of angiogenesis therapy and long-term effects of angiogenesis therapy. The acute side effect of VEGF protein or gene would include hypotension due to the upregulation of nitric oxide synthase (Hariawala et al. 1996; Horowitz et al. 1997) and increase vessel permeability. No major case of hypotension were reported in both animal studies and clinical trial, local and only transient and local edema were observed (Baumgartner et al. 2000) (Rajagopalan et al. 2003). There are three important theoretical long-term concerns of therapeutic angiogenesis. First, there is a significant concern that the induction of angiogenesis may increase the risk of neoplastic disease and malignancy. This concern arises from the fact that angiogenic cytokines were initially discovered as factors that regulate vascularization of tumors (Folkman 1971).

The induction or worsening of retinopathy has also been a legitimate concern in clinical trials of therapeutic angiogenesis. Patients with proliferative retinopathy have been shown to have high levels of VEGF in their ocular fluid (Aiello et al. 1994) suggesting that VEGF may stimulate retinal neovascularization. Despite this finding, clinical trials of therapeutic angiogenesis have not demonstrated an untoward effect of angiogenic cytokines on the

development of retinopathy. A third concern of therapeutic angiogenesis for vascular disease is that the induction of angiogenesis may stimulate the development and progression of atherosclerotic plaques that may lead to unstable vascular syndromes but no increase in adverse cardiovascular events in patients treated with angiogenic cytokines compared to control patients.

Potential adverse acute effects may be associated with the means of delivery of the agent. Surgical and percutaneous techniques all carry risk of morbidity and mortality. Clinical trials for therapeutic angiogenesis have shown some incidence of peri-procedural adverse events in patients undergoing procedures for the delivery of angiogenic cytokines, however these have appeared to be device related and not a result of the therapeutic. Surgical delivery of angiogenic agents is generally simpler than standard cardiac surgical procedures. With adequate training, the percutaneous route of delivery using NOGA is extremely safe with rare occurrences of severe complications.

At the present time, the ideal angiogenic agent, the optimal preparation of that agent, and the optimal delivery route of the agent remain uncertain and continues to be a topic of considerable debate. Ongoing and future trials will attempt to identify the combination of these variables that will provide for the safest and most efficacious route of achieving therapeutic neovascularization.

E. Summary

As the population continues to age, there will continue to be growth in the burden of ischemic cardiovascular disease. At the present time, no form of therapy has proven beyond doubt to be effective in treating this patient population who has no other options for conventional revascularization therapies. A large body of data has now been accumulated that suggests that therapeutic angiogenesis may offer hope for this patient population.

The data regarding therapeutic angiogenesis presented in this chapter show that most presently available methods for inducing therapeutic angiogenesis appear safe and feasible. No clear safety issues have been identified with the induction of angiogenesis and delivery strategies are becoming less invasive thereby reducing procedural risks to patients. Despite safety and feasibility of these strategies, the efficacy of therapeutic angiogenesis has not been definitively proven in Phase I and Phase II studies that have been performed thus far.

The field of therapeutic angiogenesis now depends on the performance of large Phase III randomized placebo-controlled trials to better understand therapeutic angiogenesis and to determine the most optimal means of achieving therapeutic angiogenesis. These studies face several challenges prior to their inception. First, the ideal agent for the induction of therapeutic angiogenesis needs to be identified. VEGF, FGF, and progenitor cells have been most actively studied but future studies are being planned on other potential angiogenic factors such as hepatocyte growth factor, sonic hedgehog and others. Additionally, recent pre-clinical data suggests that a combination of progenitor cells with angiogenic cytokines may be more effective at inducing angiogenesis (Kawamoto et al. 2004). Second, the ideal form of the angiogenic cytokine needs to be determined. Considerable debate remains regarding whether protein, naked DNA or viral encoded gene is the safest and most efficacious mean of inducing therapeutic angiogenesis. Third, the optimal dose of each of the potential angiogenic agents remains to be identified.

Important challenges remain in terms of design of clinical trials of therapeutic angiogenesis. It is vital that future trials have control/placebo groups as Phase I and II studies have shown the significant placebo effect in patients undergoing control therapy. Control groups are also vitally important to assess safety of angiogenic therapy. An understanding of the occurrence

of malignancies and retinopathy with angiogenic therapy can only be assessed with a knowledge of the occurrence of these adverse events in a control group. Finally, consensus needs to be established regarding the best clinical endpoint to use to assess efficacy and the ideal time point to assess these endpoints.

References

- Aiello LP, Avery RL, et al. Vascular endothelial growth factor in ocular fluids of patients with diabetic retinopathy and other retinal disorders. *New England Journal of Medicine* 1994;331:1480–1487. [PubMed: 7526212]
- Archundia A, Aceves JL, et al. Direct cardiac injection of G-CSF mobilized bone-marrow stem-cells improves ventricular function in old myocardial infarction. *Life Sci* 2005;78(3):279–83. [PubMed: 16183081]
- Asahara T, Masuda H, et al. Bone marrow origin of endothelial progenitor cells responsible for postnatal vasculogenesis in physiological and pathological neovascularization. *Circ Res* 1999;85(3):221–8. [PubMed: 10436164]
- Asahara T, Murohara T, et al. Isolation of putative progenitor endothelial cells for angiogenesis. *Science* 1997;275(5302):964–7. [PubMed: 9020076]
- Askari AT, Unzek S, et al. Effect of stromal-cell-derived factor 1 on stem-cell homing and tissue regeneration in ischaemic cardiomyopathy. *Lancet* 2003;362(9385):697–703. [PubMed: 12957092]
- Assmus B, Honold J, et al. Transcatheter transplantation of progenitor cells after myocardial infarction. *N Engl J Med* 2006;355(12):1222–32. [PubMed: 16990385]
- Assmus B, Schachinger V, et al. Transplantation of Progenitor Cells and Regeneration Enhancement in Acute Myocardial Infarction (TOPCARE-AMI). *Circulation* 2002;106(24):3009–17. [PubMed: 12473544]
- Azarnoush K, Maurel A, et al. Enhancement of the functional benefits of skeletal myoblast transplantation by means of coadministration of hypoxia-inducible factor 1alpha. *J Thorac Cardiovasc Surg* 2005;130(1):173–9. [PubMed: 15999059]
- Banai S, Jaklitsch MT, et al. Angiogenic-induced enhancement of collateral blood flow to ischemic myocardium by vascular endothelial growth factor in dogs. *Circulation* 1994;89:2183–2189. [PubMed: 7514110]
- Bartunek J, Vanderheyden M, et al. Intracoronary injection of CD133-positive enriched bone marrow progenitor cells promotes cardiac recovery after recent myocardial infarction: feasibility and safety. *Circulation* 2005;112(9 Suppl):I178–83. [PubMed: 16159812]
- Baumgartner I, Rauh G, et al. Lower-extremity edema associated with gene transfer of naked DNA vascular endothelial growth factor. *Annals of Internal Medicine* 2000;132:880–884. [PubMed: 10836914]
- Bhakta S, Greco NJ, et al. The safety of autologous intracoronary stem cell injections in a porcine model of chronic myocardial ischemia. *J Invasive Cardiol* 2006;18(5):212–8. [PubMed: 16670448]
- Bokeriya LA, Golukhova EZ, et al. Use of human VEGF(165) gene for therapeutic angiogenesis in coronary patients: first results. *Bull Exp Biol Med* 2005;140(1):106–12. [PubMed: 16254634]
- Botta R, Gao E, et al. Heart infarct in NOD-SCID mice: therapeutic vasculogenesis by transplantation of human CD34+ cells and low dose CD34+KDR+ cells. *Faseb J* 2004;18(12):1392–4. [PubMed: 15231728]
- Brogi E, Schatteman G, et al. Hypoxia-induced paracrine regulation of vascular endothelial growth factor receptor expression. *J Clin Invest* 1996;97(2):469–76. [PubMed: 8567969]
- Chachques JC, Duarte F, et al. Angiogenic growth factors and/or cellular therapy for myocardial regeneration: a comparative study. *J Thorac Cardiovasc Surg* 2004;128(2):245–53. [PubMed: 15282461]
- Chen HK, Hung HF, et al. Combined cord blood stem cells and gene therapy enhances angiogenesis and improves cardiac performance in mouse after acute myocardial infarction. *Eur J Clin Invest* 2005;35(11):677–86. [PubMed: 16269017]

- Chen SL, Fang WW, et al. Effect on left ventricular function of intracoronary transplantation of autologous bone marrow mesenchymal stem cell in patients with acute myocardial infarction. *Am J Cardiol* 2004;94(1):92–5. [PubMed: 15219514]
- Coussens LM, Tinkle CL, et al. MMP-9 supplied by bone marrow-derived cells contributes to skin carcinogenesis. *Cell* 2000;103(3):481–90. [PubMed: 11081634]
- Cuevas P, Carceller F, et al. Hypotensive activity of fibroblast growth factor. *Science* 1991;254:1208–1210. [PubMed: 1957172]
- Erbs S, Linke A, et al. Transplantation of blood-derived progenitor cells after recanalization of chronic coronary artery occlusion: first randomized and placebo-controlled study. *Circ Res* 2005;97(8):756–62. [PubMed: 16151021]
- Folkman J. Tumor angiogenesis: therapeutic implications. *New England Journal of Medicine* 1971;285:1182–1186. [PubMed: 4938153]
- Fuchs S, Baffour R, et al. Transendocardial delivery of autologous bone marrow enhances collateral perfusion and regional function in pigs with chronic experimental myocardial ischemia. *J Am Coll Cardiol* 2001;37(6):1726–32. [PubMed: 11345391]
- Gal D, Weir L, et al. Direct myocardial transfection in two animal models: Evaluation of parameters affecting gene expression and percutaneous gene delivery. *Laboratory Investigation* 1993;68:18–25. [PubMed: 8423672]
- Giordano FJ, Ping P, et al. Intracoronary gene transfer of fibroblast growth factor-5 increases blood flow and contractile function in an ischemic region of the heart. *Nat Med* 1996;2(5):534–9. [PubMed: 8616711]
- Goldberg MA, Schneider TJ. Similarities between the oxygen-sensing mechanisms regulating the expression of vascular endothelial growth factor and erythropoietin. *Journal of Biological Chemistry* 1994;269:4355–4359. [PubMed: 8308005]
- Grines CL, Watkins MW, et al. Angiogenic Gene Therapy (AGENT) trial in patients with stable angina pectoris. *Circulation* 2002;105(11):1291–7. [PubMed: 11901038]
- Grines CL, Watkins MW, et al. A randomized, double-blind, placebo-controlled trial of Ad5FGF-4 gene therapy and its effect on myocardial perfusion in patients with stable angina. *J Am Coll Cardiol* 2003;42(8):1339–47. [PubMed: 14563572]
- Gyongyosi M, Khorsand A, et al. 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(9 Suppl):I157–65. [PubMed: 16159809]
- Harada K, Friedman M, et al. Vascular endothelial growth factor in chronic myocardial ischemia. *American Journal of Physiology* 1996;270:H1791–H1802. [PubMed: 8928888]
- Hariawala MD, Horowitz JR, et al. VEGF improves myocardial blood flow but produces EDRF-mediated hypotension in porcine hearts. *J Surg Res* 1996;63(1):77–82. [PubMed: 8661176]
- Hedman M, Hartikainen J, et al. Safety and feasibility of catheter-based local intracoronary VEGF transfer in the prevention of postangioplasty and in-stent restenosis and in the treatment of chronic myocardial ischemia. Phase II results of the KAT-trial. *Circulation*. 2003
- Hendrikx M, Hensen K, et al. Recovery of regional but not global contractile function by the direct intramyocardial autologous bone marrow transplantation: results from a randomized controlled clinical trial. *Circulation* 2006;114(1 Suppl):I101–7. [PubMed: 16820557]
- Henry TD, Abraham JA. Review of preclinical and clinical results with vascular endothelial growth factors for therapeutic angiogenesis. *Current Interventional Cardiology Reports* 2000;2:228–241. [PubMed: 11096672]
- Henry TD, Annex BH, et al. The VIVA trial: Vascular endothelial growth factor in ischemia or vascular angiogenesis. *Circulation* 2003;107(10):1359–1365. [PubMed: 12642354]
- Henry TD, Rocha-Sing K, et al. Intracoronary administration of recombinant human vascular endothelial growth factor (rhVEGF) to patients with coronary artery disease. *American Heart Journal*. 2001
- Horowitz JR, Rivard A, et al. Vascular endothelial growth factor/vascular permeability factor produces nitric oxide-dependent hypotension. *Arteriosclerosis and Thrombosis and Vascular Biology* 1997;17:2793–2800.

- Huang YQ, Li JJ, et al. Identification of a family of alternatively spliced mRNA species of angiopoietin-1. *Blood* 2000;95(6):1993–9. [PubMed: 10706866]
- Ince H, Petzsch M, et al. 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(9 Suppl):I73–80. [PubMed: 16159869]
- Iwaguro H, Yamaguchi J, et al. Endothelial progenitor cell vascular endothelial growth factor gene transfer for vascular regeneration. *Circulation* 2002;105(6):732–8. [PubMed: 11839630]
- Janssens S, Dubois C, et al. Autologous bone marrow-derived stem-cell transfer in patients with ST-segment elevation myocardial infarction: double-blind, randomised controlled trial. *Lancet* 2006;367(9505):113–21. [PubMed: 16413875]
- Kalka C, Masuda H, et al. Vascular endothelial growth factor(165) gene transfer augments circulating endothelial progenitor cells in human subjects. *Circ Res* 2000;86(12):1198–202. [PubMed: 10864908]
- Kamihata H, Matsubara H, et al. Implantation of bone marrow mononuclear cells into ischemic myocardium enhances collateral perfusion and regional function via side supply of angioblasts, angiogenic ligands, and cytokines. *Circulation* 2001;104(9):1046–52. [PubMed: 11524400]
- Kang HJ, Kim HS, et al. 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(9411):751–6. [PubMed: 15016484]
- Kang HJ, Lee HY, et al. Differential effect of intracoronary infusion of mobilized peripheral blood stem cells by granulocyte colony-stimulating factor on left ventricular function and remodeling in patients with acute myocardial infarction versus old myocardial infarction: the randomized, controlled trial. *Circulation* 2006;114(1 Suppl):I145–51. [PubMed: 16820564]
- Kastrup J, Jorgensen E, et al. 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(7):982–8. [PubMed: 15808751]
- Katrtsis DG, Sotiropoulou PA, et al. Transcoronary transplantation of autologous mesenchymal stem cells and endothelial progenitors into infarcted human myocardium. *Catheter Cardiovasc Interv* 2005;65(3):321–9. [PubMed: 15954106]
- Kawamoto A, Gwon HC, et al. Therapeutic potential of ex vivo expanded endothelial progenitor cells for myocardial ischemia. *Circulation* 2001;103(5):634–7. [PubMed: 11156872]
- Kawamoto A, Murayama T, et al. Synergistic effect of bone marrow mobilization and vascular endothelial growth factor-2 gene therapy in myocardial ischemia. *Circulation* 2004;110(11):1398–405. [PubMed: 15337699]
- Kinnaird T, Stabile E, et al. Marrow-derived stromal cells express genes encoding a broad spectrum of arteriogenic cytokines and promote in vitro and in vivo arteriogenesis through paracrine mechanisms. *Circ Res* 2004;94(5):678–85. [PubMed: 14739163]
- Kinnaird T, Stabile E, et al. Local delivery of marrow-derived stromal cells augments collateral perfusion through paracrine mechanisms. *Circulation* 2004;109(12):1543–9. [PubMed: 15023891]
- Kocher AA, Schuster MD, et al. Neovascularization of ischemic myocardium by human bone-marrow-derived angioblasts prevents cardiomyocyte apoptosis, reduces remodeling and improves cardiac function. *Nat Med* 2001;7(4):430–6. [PubMed: 11283669]
- Kolsut P, Malecki M, et al. Gene therapy of coronary artery disease with phvegf165--early outcome. *Kardiol Pol* 2003;59(11):373–84. [PubMed: 14668888]
- Kueth F, Figulla HR, et al. Mobilization of stem cells by granulocyte colony-stimulating factor for the regeneration of myocardial tissue after myocardial infarction. *Dtsch Med Wochenschr* 2004;129(9):424–8. [PubMed: 14970913]
- Kupatt C, Horstkotte J, et al. Embryonic endothelial progenitor cells expressing a broad range of proangiogenic and remodeling factors enhance vascularization and tissue recovery in acute and chronic ischemia. *Faseb J* 2005;19(11):1576–8. [PubMed: 16009705]

- Laham RJ, Chronos NA, et al. Intracoronary basic fibroblast growth factor (FGF-2) in patients with severe ischemic heart disease: results of a phase 1 open-label dose escalation study. *Journal of the American College of Cardiology* 2000;36:2132–2139. [PubMed: 11127452]
- Laham RJ, Sellke FW, et al. Local perivascular delivery of basic fibroblast growth factor in patients undergoing coronary bypass surgery: results of a phase 1 randomized, double-blind, placebo-controlled trial. *Circulation* 1999;100:1865–1871. [PubMed: 10545430]
- Lazarous DF, Scheinowitz M, et al. Effects of chronic systemic administration of basic fibroblast growth factor on collateral development in the canine heart. *Circulation* 1995;91:145–153. [PubMed: 7805195]
- Lazarous DF, Shou M, et al. Adenoviral-mediated gene transfer induces sustained pericardial VEGF expression in dogs: effect on myocardial angiogenesis. *Cardiovascular Research* 1999;44:294–302. [PubMed: 10690306]
- Lee LY, Patel SR, et al. Focal angiogen therapy using intramyocardial delivery of an adenovirus vector coding for vascular endothelial growth factor 121. *Ann Thorac Surg* 2000;69(1):14–23. [PubMed: 10654479]discussion 23–4
- Levy AP, Levy NS, et al. Post-transcriptional regulation of vascular endothelial growth factor by hypoxia. *Journal of Biological Chemistry* 1996;371:2746–2753. [PubMed: 8576250]
- Liu TB, Fedak PW, et al. Enhanced IGF-1 expression improves smooth muscle cell engraftment after cell transplantation. *Am J Physiol Heart Circ Physiol* 2004;287(6):H2840–9. [PubMed: 15331370]
- Liu Y, Guo J, et al. Bone marrow mononuclear cell transplantation into heart elevates the expression of angiogenic factors. *Microvasc Res* 2004;68(3):156–60. [PubMed: 15501234]
- Lopez JJ, Laham RJ, et al. VEGF administration in chronic myocardial ischemia in pigs. *Cardiovascular Research* 1998;40:272–281. [PubMed: 9893720]
- Losordo DW. Intramyocardial Transplantation of Autologous CD34+ Stem Cells for Intractable Angina: A Phase I/IIa Double-Blind Randomized Controlled Trial. *Circulation*. 2007In Press
- Losordo DW, Pickering JG, et al. Use of the rabbit ear artery to serially assess foreign protein secretion after site specific arterial gene transfer in vivo: Evidence that anatomic identification of successful gene transfer may underestimate the potential magnitude of transgene expression. *Circulation* 1994;89:785–792. [PubMed: 8313567]
- Losordo DW, Vale PR, et al. 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(17):2012–8. [PubMed: 11980678]
- Losordo DW, Vale PR, et al. Gene therapy for myocardial angiogenesis: initial clinical results with direct myocardial injection of phVEGF165 as sole therapy for myocardial ischemia. *Circulation* 1998;98(25):2800–4. [PubMed: 9860779]
- Lunde K, Solheim S, et al. Intracoronary injection of mononuclear bone marrow cells in acute myocardial infarction. *N Engl J Med* 2006;355(12):1199–209. [PubMed: 16990383]
- Lunde K, Solheim S, et al. Autologous stem cell transplantation in acute myocardial infarction: The ASTAMI randomized controlled trial. Intracoronary transplantation of autologous mononuclear bone marrow cells, study design and safety aspects. *Scand Cardiovasc J* 2005;39(3):150–8. [PubMed: 16152682]
- Luttun A, Tjwa M, et al. Revascularization of ischemic tissues by PlGF treatment, and inhibition of tumor angiogenesis, arthritis and atherosclerosis by anti-Flt1. *Nat Med* 2002;8(8):831–40. [PubMed: 12091877]
- Ma J, Ge J, et al. Time course of myocardial stromal cell-derived factor 1 expression and beneficial effects of intravenously administered bone marrow stem cells in rats with experimental myocardial infarction. *Basic Res Cardiol* 2005;100(3):217–23. [PubMed: 15754085]
- Mack CA, Patel SR, et al. Biologic bypass with the use of adenovirus-mediated gene transfer of the complementary deoxyribonucleic acid for vascular endothelial growth factor 121 improves myocardial perfusion and function in the ischemic porcine heart. *J Thorac Cardiovasc Surg* 1998;115(1):168–76. [PubMed: 9451061]discussion 176–7
- Matsumoto R, Omura T, et al. Vascular endothelial growth factor-expressing mesenchymal stem cell transplantation for the treatment of acute myocardial infarction. *Arterioscler Thromb Vasc Biol* 2005;25(6):1168–73. [PubMed: 15831811]

- Meyer GP, Wollert KC, et al. Intracoronary bone marrow cell transfer after myocardial infarction: eighteen months' follow-up data from the randomized, controlled BOOST (BOne marrOW transfer to enhance ST-elevation infarct regeneration) trial. *Circulation* 2006;113(10):1287–94. [PubMed: 16520413]
- Moon MH, Kim SY, et al. Human adipose tissue-derived mesenchymal stem cells improve postnatal neovascularization in a mouse model of hindlimb ischemia. *Cell Physiol Biochem* 2006;17(56):279–90. [PubMed: 16791003]
- Murohara T, Ikeda H, et al. Transplanted cord blood-derived endothelial precursor cells augment postnatal neovascularization. *J Clin Invest* 2000;105(11):1527–36. [PubMed: 10841511]
- Nagaya N, Fujii T, et al. Intravenous administration of mesenchymal stem cells improves cardiac function in rats with acute myocardial infarction through angiogenesis and myogenesis. *Am J Physiol Heart Circ Physiol* 2004;287(6):H2670–6. [PubMed: 15284059]
- Numaguchi Y, Sone T, et al. The impact of the capability of circulating progenitor cell to differentiate on myocardial salvage in patients with primary acute myocardial infarction. *Circulation* 2006;114(1 Suppl):I114–9. [PubMed: 16820559]
- Ohnishi S, Yanagawa B, et al. Transplantation of mesenchymal stem cells attenuates myocardial injury and dysfunction in a rat model of acute myocarditis. *J Mol Cell Cardiol* 2007;42(1):88–97. [PubMed: 17101147]
- Olivares EL, Ribeiro VP, et al. Bone marrow stromal cells improve cardiac performance in healed infarcted rat hearts. *Am J Physiol Heart Circ Physiol* 2004;287(2):H464–70. [PubMed: 15044198]
- Orlic D, Kajstura J, et al. Mobilized bone marrow cells repair the infarcted heart, improving function and survival. *Proc Natl Acad Sci U S A* 2001;98(18):10344–9. [PubMed: 11504914]
- Pearlman JD, Hibberd MG, et al. Magnetic resonance mapping demonstrates benefits of VEGF-induced myocardial angiogenesis. *Nature Medicine* 1995;1:1085–1089.
- Perin EC, Dohmann HF, et al. Transendocardial, autologous bone marrow cell transplantation for severe, chronic ischemic heart failure. *Circulation* 2003;107(18):2294–302. [PubMed: 12707230]
- Pittenger MF, Mackay AM, et al. Multilineage potential of adult human mesenchymal stem cells. *Science* 1999;284(5411):143–7. [PubMed: 10102814]
- Pompilio G, Cannata A, et al. Autologous peripheral blood stem cell transplantation for myocardial regeneration: a novel strategy for cell collection and surgical injection. *Ann Thorac Surg* 2004;78(5):1808–12. [PubMed: 15511478]
- Prockop DJ. Marrow stromal cells as stem cells for nonhematopoietic tissues. *Science* 1997;276(5309):71–4. [PubMed: 9082988]
- Raffi S. Circulating endothelial precursors: mystery, reality, and promise. *J Clin Invest* 2000;105(1):17–9. [PubMed: 10619857]
- Raffi S, Heissig B, et al. Efficient mobilization and recruitment of marrow-derived endothelial and hematopoietic stem cells by adenoviral vectors expressing angiogenic factors. *Gene Ther* 2002;9(10):631–41. [PubMed: 12032709]
- Rajagopalan S, Mohler ER 3rd, et al. Regional angiogenesis with vascular endothelial growth factor in peripheral arterial disease: a phase II randomized, double-blind, controlled study of adenoviral delivery of vascular endothelial growth factor 121 in patients with disabling intermittent claudication. *Circulation* 2003;108(16):1933–8. [PubMed: 14504183]
- Ripa RS, Jorgensen E, et al. 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 stem cells in myocardial infarction (STEMMI) trial. *Circulation* 2006;113(16):1983–92. [PubMed: 16531621]
- Rosengart TK, Lee LY, et al. Angiogenesis gene therapy: Phase I assessment of direct intramyocardial administration of an adenovirus vector expression VEGF121 cDNA to individuals with clinically significant severe coronary artery disease. *Circulation* 1999;100:468–474. [PubMed: 10430759]
- Ryabov VV, Krylov AL, et al. Cardiac contractility after transplantation of autologous mononuclear bone marrow cells in patients with myocardial infarction. *Bull Exp Biol Med* 2006;141(1):124–8. [PubMed: 16929983]

- Schachinger V, Assmus B, et al. Transplantation of progenitor cells and regeneration enhancement in acute myocardial infarction: final one-year results of the TOPCARE-AMI Trial. *J Am Coll Cardiol* 2004;44(8):1690–9. [PubMed: 15489105]
- Schachinger V, Erbs S, et al. Intracoronary bone marrow-derived progenitor cells in acute myocardial infarction. *N Engl J Med* 2006;355(12):1210–21. [PubMed: 16990384]
- Schaefer A, Meyer GP, et al. Impact of intracoronary bone marrow cell transfer on diastolic function in patients after acute myocardial infarction: results from the BOOST trial. *Eur Heart J* 2006;27(8):929–35. [PubMed: 16510465]
- Schuh A, Breuer S, et al. Administration of vascular endothelial growth factor adjunctive to fetal cardiomyocyte transplantation and improvement of cardiac function in the rat model. *J Cardiovasc Pharmacol Ther* 2005;10(1):55–66. [PubMed: 15821839]
- Schumacher B, Stegmann T, et al. The stimulation of neoangiogenesis in the ischemic human heart by the growth factor FGF: first clinical results. *Journal of Cardiovascular Surgery* 1998;39:783–789. [PubMed: 9972900]
- Seiler C, Pohl T, et al. 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(17):2012–7. [PubMed: 11673338]
- Sellke FW, Laham RJ, et al. Therapeutic angiogenesis with basic fibroblast growth factor: technique and early results. *Annals of Thoracic Surgery* 1998;65:1540–1544. [PubMed: 9647055]
- Shi Q, Rafii S, et al. Evidence for circulating bone marrow-derived endothelial cells. *Blood* 1998;92(2):362–7. [PubMed: 9657732]
- Shintani S, Kusano K, et al. Synergistic effect of combined intramyocardial CD34(+) cells and VEGF2 gene therapy after MI. *Nat Clin Pract Cardiovasc Med* 2006;3(Suppl 1S1):S123–S128. [PubMed: 16501618]
- Shyu KG, Wang BW, et al. Mesenchymal stem cells are superior to angiogenic growth factor genes for improving myocardial performance in the mouse model of acute myocardial infarction. *J Biomed Sci* 2006;13(1):47–58. [PubMed: 16283432]
- Silva GV, Litovsky S, et al. Mesenchymal stem cells differentiate into an endothelial phenotype, enhance vascular density, and improve heart function in a canine chronic ischemia model. *Circulation* 2005;111(2):150–6. [PubMed: 15642764]
- Simons M, Annex BH, et al. Pharmacological treatment of coronary artery disease with recombinant fibroblast growth factor-2: double-blind, randomized, controlled clinical trial. *Circulation* 2002;105(7):788–93. [PubMed: 11854116]
- Stamm C, Westphal B, et al. Autologous bone-marrow stem-cell transplantation for myocardial regeneration. *Lancet* 2003;361(9351):45–6. [PubMed: 12517467]
- Stegmann TJ, Hoppert T, et al. First angiogenic treatment of coronary heart disease by FGF-1: long-term results after 3 years. *Cardiac and Vascular Regeneration* 2000;1:5–10.
- Stegmann TJ, Hoppert T, et al. Induction of myocardial neoangiogenesis by human growth factors. A new therapeutic option in coronary heart disease. *Herz* 2000;25:589–599. [PubMed: 11076317]
- Steinwender C, Hofmann R, et al. Effects of peripheral blood stem cell mobilization with granulocyte-colony stimulating factor and their transcatheter transplantation after primary stent implantation for acute myocardial infarction. *Am Heart J* 2006;151(6):1296 e7–13. [PubMed: 16781240]
- Stewart JD. A phase 2 randomized, multicenter, 26-week study to assess the efficacy and safety of BIOBYPASS (adgfVEGF121.10) delivered through maximally invasive surgery versus maximal medical treatment in patients with severe angina, advanced coronary artery disease and no options for revascularization. *Circulation* 2002;106:2986–a. [PubMed: 12460884]
- Strauer BE, Brehm M, et al. Repair of infarcted myocardium by autologous intracoronary mononuclear bone marrow cell transplantation in humans. *Circulation* 2002;106(15):1913–8. [PubMed: 12370212]
- Suzuki K, Nagashima K, et al. Effect of granulocyte colony-stimulating factor treatment at a low dose but for a long duration in patients with coronary heart disease. *Circ J* 2006;70(4):430–7. [PubMed: 16565560]

- Symes JF, Losordo DW, et al. Gene therapy with vascular endothelial growth factor for inoperable coronary artery disease: preliminary clinical results. *Annals of Thoracic Surgery* 1999;68:830–837. [PubMed: 10509970]
- Takahashi M, Li TS, et al. 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(2):H886–93. [PubMed: 16603697]
- Takahashi T, Kalka C, et al. Ischemia- and cytokine-induced mobilization of bone marrow-derived endothelial progenitor cells for neovascularization. *Nat Med* 1999;5(4):434–8. [PubMed: 10202935]
- Takeshita S, Isshiki T, et al. Increased expression of direct gene transfer into skeletal muscles observed after acute ischemic injury in rats. *Laboratory Investigation* 1996;74:1061–1065. [PubMed: 8667610]
- Takeshita S, Losordo DW, et al. Time course of recombinant protein secretion following liposome-mediated gene transfer in a rabbit arterial organ culture model. *Laboratory Investigation* 1994;71:387–391. [PubMed: 7523763]
- Tang YL, Zhao Q, et al. Paracrine action enhances the effects of autologous mesenchymal stem cell transplantation on vascular regeneration in rat model of myocardial infarction. *Ann Thorac Surg* 2005;80(1):229–36. [PubMed: 15975372]discussion 236–7
- Tio RA, Tan ES, et al. PET for evaluation of differential myocardial perfusion dynamics after VEGF gene therapy and laser therapy in end-stage coronary artery disease. *J Nucl Med* 2004;45(9):1437–43. [PubMed: 15347709]
- Tse HF, Kwong YL, et al. Angiogenesis in ischaemic myocardium by intramyocardial autologous bone marrow mononuclear cell implantation. *Lancet* 2003;361(9351):47–9. [PubMed: 12517468]
- Tsurumi Y, Takeshita S, et al. Direct intramuscular gene transfer of naked DNA encoding vascular endothelial growth factor augments collateral development and tissue perfusion. *Circulation* 1996;94:3281–3290. [PubMed: 8989142]
- Udelson JE, Dilsizian V, et al. 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;102:1605–1610. [PubMed: 11015335]
- Unger EF, Banai S, et al. Basic fibroblast growth factor enhances myocardial collateral flow in a canine model. *American Journal of Physiology* 1994;266:H1588–H1595. [PubMed: 8184938]
- Unger EF, Goncalves L, et al. Effects of a single intracoronary injection of basic fibroblast growth factor in stable angina pectoris. *Am J Cardiol* 2000;85(12):1414–1419. [PubMed: 10856385]
- Vale PR, Losordo DW, et al. Left ventricular electromechanical mapping to assess efficacy of phVEGF (165) gene transfer for therapeutic angiogenesis in chronic myocardial ischemia. *Circulation* 2000;102(9):965–74. [PubMed: 10961959]
- Vale PR, Losordo DW, et al. 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(17):2138–43. [PubMed: 11331253]
- Vale PR, Losordo DW, et al. Catheter-based myocardial gene transfer utilizing nonfluoroscopic electromechanical left ventricular mapping. *J Am Coll Cardiol* 1999;34(1):246–54. [PubMed: 10400018]
- Valgimigli M, Rigolin GM, et al. 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(18):1838–45. [PubMed: 15860518]
- Waltenberger J, Mayr U, et al. Functional upregulation of the vascular endothelial growth factor receptor KDR by hypoxia. *Circulation* 1996;94:1647–1654. [PubMed: 8840857]
- Wartiovaara U, Salven P, et al. Peripheral blood platelets express VEGF-C and VEGF which are released during platelet activation. *Thromb Haemost* 1998;80(1):171–5. [PubMed: 9684805]
- Wolff JA, Malone RW, et al. Direct gene transfer into mouse muscle in vivo. *Science* 1990;247:1465–1468. [PubMed: 1690918]

- Wollert KC, Meyer GP, et al. Intracoronary autologous bone-marrow cell transfer after myocardial infarction: the BOOST randomised controlled clinical trial. *Lancet* 2004;364(9429):141–8. [PubMed: 15246726]
- Yamaguchi J, Kusano KF, et al. Stromal cell-derived factor-1 effects on ex vivo expanded endothelial progenitor cell recruitment for ischemic neovascularization. *Circulation* 2003;107(9):1322–8. [PubMed: 12628955]
- Yang J, Zhou W, et al. Effects of Myocardial Transplantation of Marrow Mesenchymal Stem Cells Transfected with Vascular Endothelial Growth Factor for the Improvement of Heart Function and Angiogenesis after Myocardial Infarction. *Cardiology* 2006;107(1):17–29. [PubMed: 16741354]
- Yaoita H, Takase S, et al. Scintigraphic assessment of the effects of bone marrow-derived mononuclear cell transplantation combined with off-pump coronary artery bypass surgery in patients with ischemic heart disease. *J Nucl Med* 2005;46(10):1610–7. [PubMed: 16204710]
- Yau TM, Kim C, et al. Maximizing ventricular function with multimodal cell-based gene therapy. *Circulation* 2005;112(9 Suppl):I123–8. [PubMed: 16159803]
- Zhang S, Jia Z, et al. Purified human bone marrow multipotent mesenchymal stem cells regenerate infarcted myocardium in experimental rats. *Cell Transplant* 2005;14(10):787–98. [PubMed: 16454353]
- Zohlhofer D, Ott I, et al. Stem cell mobilization by granulocyte colony-stimulating factor in patients with acute myocardial infarction: a randomized controlled trial. *Jama* 2006;295(9):1003–10. [PubMed: 16507801]

Table 1

Clinical Trials of protein/gene therapy for Coronary Artery Disease

rVEGF-recombinant vascular endothelial growth factor; CMI- chronic myocardial ischemia; NO- no option for revascularization; IC- intracoronary; SPECT- single positron emission computed tomography; CCS- Canadian Cardiovascular Society angina class; IV- intravenous; WT- walking time; phVEGF₁₆₅- plasmid encoding vascular endothelial growth factor 165; IMy- intramyocardial; THthoracotomy; phVEGF2- plasmid encoding vascular endothelial growth factor 2; NTG- nitroglycerin; QOL- quality of life; AdVEGF₁₂₁- adenovirus encoding vascular endothelial growth factor 121; WMS-wall motion score; TTI- time to ischemia; FGF- fibroblast growth factor; PA- periaortic; CA- coronary angiography; rVEGF- recombinant basic fibroblast growth factor; EF- ejection fraction; AdFGF5- adenovirus encoding FGF5; CAD- coronary artery disease; CABG- coronary artery bypass grafting; rFGF2- recombinant protein FGF2; rVEGF- recombinant protein VEGF; PCI- percutaneous coronary intervention; EP- endpoint

Reference	Therapy	Patient Characteristics	Delivery Route	Phase	Active/Placebo		Findings
					n/n		
Henry (Henry and Abraham 2000)	rVEGF	CMI-NO	IV	I	28/0		Improved SPECT, collaterals
Henry (Henry et al. 2001)	rVEGF	CMI-NO	IC	I	15/0		Improved SPECT, CCS
VIVA (Henry et al. 2003)	rVEGF	CMI-NO	IC/IV	II	115/63		No DWT at 60 days
Selke (Selke et al. 1998)	rFGF2	CMI	PA during CABG	I	8/0		Improved SPECT, EF
Schumacher (Schumacher et al. 1998)	rFGF	CMI	PA during CABG	I	20/0		Increased capillaries on CA
Laham (Laham et al. 1999)	rFGF2	CMI	PA during CABG	I	16/8		Improved SPECT in high dose group
Udelson (Udelson et al. 2000)	rFGF2	CMI-NO	IC or IV	I	59/0		Improved stress/rest SPECT
Laham (Laham et al. 2000)	rFGF2	CMI-NO	IC	I	52/0		Improved QOL, DWT; WMS
Unger (Unger et al. 2000)	rFGF2	CMI	IC	I	17/8		No DWT
FIRST (Simons et al. 2002)	rFGF2	CMI-NO	IC	II	251/86		No difference in DWT
Vale (Vale et al. 2001)	phVEGF2	CMI-NO	IMy-NOGA	I	3/3		Improved angina, NTG use
Losordo (Losordo et al. 2002)	phVEGF2	CMI-NO	IMy-NOGA	I/II	13/6		Improved CCS, DWT, QOL
GENASES*	phVEGF-2	CMI-NO	IMy-Stiletto	IIb	300/100		Primary EP: DWT at 3 months
Tio (Tio et al. 2004)	phVEGF165	end stage CAD				VEGF/laser/standard 10/12/13	improved perfusion in the VEGF group
Symes (Symes et al. 1999)	phVEGF165	CMI-NO	IMy-TH	I	30/0		Improved SPECT, angina, DWT
Vale (Vale et al. 2000)	phVEGF 165		IMy	I	13/0		
Losordo (Losordo et al. 1998)	phVEGF 165		IMy-TH	I	5/0		
Kolsut (Kolsut et al. 2003)	phVEGF165	MI with CABG	IMy			CABG 14/no CABG 8	improved left ventricular functions
Euroinject One trial (Kastrup et al. 2005)	phVEGF165	MI without CABG					No improvement

Reference	Therapy	Patient Characteristics	Delivery Route	Phase	Active/Placebo		Findings
						n/n	
Euroinject I (Gyongyosi et al. 2005) Euroinject I (Bokeriya et al. 2005)	phVEGF165	CMI-NO	IMy-NOGA	II		40/40	Improved SPECT, WMS
	phVEGF165	CMI-NO	IMy-NOGA	II		40/40	
	phVEGF165						
Rosengart (Rosengart et al. 1999) REVASC (Stewart 2002) Hedman (Hedman et al. 2003)	AdVEGF121	CMI±NO	IMy-TH ± CABG	I		21/0	Improved CCS, collaterals, WMS
	AdVEGF121	CMI-NO	IMy-TH	II		34/33	Significant improvement TTI, QOL
	AdVEGF/ phVEGF	CMI for PCI	IC	II		37/28/38	No D in angio/clinical restenosis
AGENT (Grines et al. 2002) AGENT 3, 2004 Grines (Grimes et al. 2003)	AdFGF5	CMI	IC	I		60/19	Trend to increased WT at 4 weeks
	AdFGF5	CMI	IC	II		234/123	No difference in DWT
	Ad5FGF-4	stable angina	IC				35/17

* denotes presently enrolling trial

Table 2

Clinical Trials of Cell Therapy for Coronary Artery Disease

G-CSF- granulocyte colony stimulating factor; BM-MNCs- bone marrow harvested mononuclear cells; CAD- coronary artery disease; CABG- coronary artery bypass grafting; IMy- intramyocardial; SPECT- single positron emission computed tomography; CMI- chronic myocardial ischemia; MRI- magnetic resonance imaging; CCS- Canadian Cardiovascular Society angina class; NO- no option for revascularization; EP- endpoint; WT- walking time; CHF- congestive heart failure; ESV- end-systolic volume; AMI- acute myocardial infarction; IC- intracoronary; IVRT- isovolumic relaxation time; DT-deceleration time; PCI- percutaneous coronary intervention; LV- left ventricular; PB-MNCs- peripherally harvested MNCs; DES- drug eluting stent; CPCs- circulating progenitor cells; CTO-chronic total occlusion; CS- left ventricular regional chordae shortening; DV- left ventricular diastolic volume; VSV- ventricular systolic volume; DD- left ventricular diastolic diameter; LVEF- left ventricular ejection fraction; MSCs- mesenchymal stem cells; EDV- left ventricular end-diastolic volume; EPCs- endothelial progenitor cells

References	Therapy		Patient Characteristics	Delivery Route	Phase	Active/Placebo		Findings
	G-CSF	Cells					n/n	
Stamm (Stamm et al. 2003)	-	BM-MNCs	CAD for CABG	IMy-CABG	I		6/0	4 patients with improved LVEF/SPECT
Tse (Tse et al. 2003)	-	BM-MNCs	CMI	IMy-NOGA	I		8/0	Improved symptoms, LVEF, perfusion on MRI
Fuchs (Fuchs et al. 2001)	-	BM-MNCs	CMI	IMy-NOGA	I		10/0	Improved CCS, stress ischemia
Perin (Perin et al. 2003)	-	BM-MNCs	CMI/CHF-NO	IMy-NOGA	I		14/7	Improved LVEF, ESV
Strauer (Strauer et al. 2002)	-	BM-MNCs	AMI	IC	I		10/10	Reduced infarct zone
BOOST trial (Wollert et al. 2004)	-	BM-MNCs	AMI	IC	II		30/30	Improved LVEF by MRI at 6 months
BOOST trial (Schaefer et al. 2006)	-	BM-MNCs	AMI				30/29	improved E/A ratio, no effect on Ea/Aa ratio, IVRT and DT
BOOST trial (Meyer et al. 2006)	-	BM-MNCs	AMI, PCI	IC (1-3 days after PCI)			30/30	no long terms effect on LV function (18 months), only effect: acceleration of LV ejection fraction recovery
Janssens, S (Janssens et al. 2006)	-	BM-MNCs	ST-elevation MI		II		33/34	reduction infarct size, improved systolic function at 4 months
Lunde, K. (Lunde et al. 2006)	-	BM-MNCs	AMI	IC (6 days after MI)			47/50	no effect
ASTAMI trial (Lunde et al. 2005)	-	BM-MNCs	AMI	IC (5-8 days after MI)			24/25	safe process
Ryabov, VV (Ryabov et al. 2006)	-	BM-MNCs	MI	IC				safe but no effects
Schachinger, V (Schachinger et al. 2006)	-	BM-MNCs	AMI	IC			102/102	improved LVEF at 4 month, general improvement at 1 year
Hendrikx, M. (Hendrikx et al. 2006)	-	BM-MNCs	for CABG				7/10	increase contractility, no difference in LVEF
MAGIC trial (Kang et al. 2004)	+	PB-MNCs	Acute and old MI	IC		G-CSF+Cell/G-CSF/ Ctrl 7/3/1		improved perfusion, LVEF but increase restenosis
Yaoita, H (Yaoita et al. 2005)	+	PB-MNCs (0,15% CD34+)	of pump CABG	IMy		10/0		Improvement of perfusion correlated with amount of CD34+ cells

References	Therapy		Patient Characteristics	Delivery Route	Phase	Active/Placebo		Findings
	G-CSF	Cells				n/n		
MAGIC Cell-3-DES trial (Kang et al. 2006) TOPCARE-AMI (Assmus et al. 2002) TOPCARE-AMI Trial (Schachinger et al. 2004) Assmus, B (Assmus et al. 2006)	+	PB-MNCs + DES BM-MNCs or PB-MNCs BM-MNCs or PB-MNCs BM-MNCs or PB-MNCs	Acute and old MI AMI AMI MI	IC IC IC	I	AMI 25/25 Old MI 16/16 20/0 BM-MNCs/PB-MNCs 29/30 no cells/PB-MNCs/BM-MNCs 23/24/28	improved LVEF and ventricular remodeling in AMI patients Improved EF and ESV increased LVEF, decreased ESV in both groups, reduced infarct syze, no cardiac hypertrophy at 12 month Improved LVEF and contractility for the BM-MNCs group	
Chen SL (Chen et al. 2004) Karitsis D. G (Karitsis et al. 2005) Erbs S (Erbs et al. 2005) Numaguchi, Y. (Numaguchi et al. 2006)	- - + -	7 day-cultured BM-MNCs (MSCs) 7 day-cultured BM-MNCs (MSCs+EPCs) 4 day-cultured PB-MNCs (CPCs) 7 day-cultured PB-MNCs (EPCs)	AMI anteroseptal MI CTO (stented) AMI (stented)	IC IC IC		34/35 11/11 12/11 26 (differentiated EPCs)/25 (nondifferentiated EPCs)	Improved LVEF, perfusion, decreased ESV, EDV improved myocardial contractility increase coronary flow, reduction infarct size, improved LVEF, (3 patients in each group add restenosis) increase myocardial salvage, decrease ESV, improved LVEF	
Pompilio, G (Pompilio et al. 2004) Bartunek J. (Bartunek et al. 2005) Archundia, A. (Archundia et al. 2005) Steinwender et al (Steinwender et al. 2006) Losordo et al *	Granocyte 34 - + + +	CD133+ cells (from PB) CD133+ cells (from BM) CD34+ cells (from PB) CD34+ cells (from PB) CD34+ cells (from PB)	MI AMI (stented) Old MI AMI CMI-NO	IMy IC Peri infarction intra stent IMy-NOGA	I I	4 19/16 5/0 30/0 16/8	safe process Improved LVEF and CS but increased restenosis Improved LVEF, DV, VSV, DD, no restenosis at 12 months improved LVEF at 6 month, increase restenosis Primary EP: DWT at 12 months	
Kueth F. (Kueth et al. 2004) FIRSTLINE-AMI Trial (Ince et al. 2005) Valgimigli, M. (Valgimigli et al. 2005) STEMMI trial (Ripa et al. 2006)	+	AMI	AMI		I	5/0	improved cardiac functions and perfusion	
	+	ST-elevation MI after PCI	ST-elevation MI after PCI		II	15/15	increased LVEF, prevented of left ventricular remodeling at 12 months	
	+	ST-elevation MI	ST-elevation MI			7/7	no side effects, increased circulating stem cells at 6 and 3 months	
	+	ST-elevation MI	ST-elevation MI			31/31	safe process but no effect	

References	Therapy		Patient Characteristics	Delivery Route	Phase	Active/Placebo n/n	Findings
	G-CSF	Cells					
Suzuki K. (Suzuki et al. 2006)	+		AMI			23/23	no serious complications at 6 month
Zohlnhofer D. (Zohlnhofer et al. 2006)	+		ST-elevation AMI			56/58	no effect, restenosis 19/54 (same as placebo)

* denotes recently completed trial