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What to do when everything has failed: alternative treatment strategies for failure revascularisations

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Abstract

Percutaneous coronary intervention and bypass grafting are effective for relieving symptoms and improving outcome in patients with coronary artery disease. Despite advances in medical treatment and revascularisation procedures, some patients with symptomatic ischaemic cardiomyopathy are not candidates for revascularisation. As life expectancy increases, interventional cardiologists and cardiac surgeons face patients with more complex disease, such as those with diffuse coronary disease that cannot be completely revascularised.

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Introduction

End-stage coronary artery disease (CAD) is characterised by severe myocardial insufficiency, often with some degree of impaired ventricular function. Conditions resulting in no-option status include diffusely and small distal vessels, recurrent in-stent restenosis, chronic total occlusions, or comorbidities that preclude the use of conventional revascularisation techniques. Patients with no options may account for up to 12% of those referred for diagnostic catheterisation.[1] In addition, an estimated 15% to 25% of patients undergoing coronary artery bypass graft (CABG) surgery will have one or more major target areas incompletely revascularised due to diffuse CAD.[2,3]

In this paper, we will discuss the alternative strategies that have been developed during the last few decades to treat symptomatic patients who do not have the option of standard therapies. We will focus on pre-clinical and clinical evidence of safety and the efficacy of stem cell therapy as a novel alternative treatment.

Alternative treatment in no-option patients

Non-conventional pharmacotherapy

A diverse group of drugs with anti-ischaemic effects may play a role in the alternative medical treatment of no-option patients.

METABOLIC MYOCARDIAL MODULATION

This term refers to a group of drugs ("partial fatty acid oxidation inhibitors") that has no effect on blood pressure, heart rate, or left ventricular systolic function, but that inhibits fatty acid metabolism and promotes glycolysis, thus making the heart more energy efficient. This group comprises four different drugs: perhexiline, etomoxir, trimetazidine, and ranolazine, however, only trimetazidine and ranolazine have been widely studied in patients with chronic angina.

In a meta-analysis of 12 clinical trials in patients with stable angina, rimetazidine reduced the frequency of angina and increased the duration of treadmill exercise.[4] Despite these encouraging findings, its potential in no-option patients has yet to be established.

Ranolazine, a new antianginal drug, has had anti-ischaemic effects in randomised trials (Table 1). Although its mechanism of action in angina remains unclear, ranolazine selectively inhibits late sodium influx and attenuates the abnormalities of ventricular repolarisation and contractility associated with ischaemia.[5] In two randomised trials comparing ranolazine and placebo, or amlodipine in patients with chronic angina, patients on ranolazine have shown increased exercise duration and clinical improvement.[6-8] Ranolazine has also been studied in patients with non-ST-elevation acute coronary syndrome in the MERLIN_TIMI 36 trial, in which 65,560 patients were randomised to receive either ranolazine or placebo. After almost 1-year follow-up, there were no significant differences in the primary endpoint (a composite of cardiovascular death, myocardial infarction, and recurrent angina). In addition, no statistically significant differences were noted in the secondary endpoints, which included all-cause death, hospital readmissions, and symptomatic documented arrhythmias.

The long-term safety of ranolazine was addressed in the ROLE (Ranolazine Open Label Experience) programme,[9] which included patients who had completed the MARISA and CARISA trials and who were willing to participate in an open-label extension. The ROLE study comprised 746 patients who were being treated with ranolazine (between 500 to 2000 mg twice daily); the follow-up was extended up to 2.8 years. The most common symptom reported was dizziness (12%), followed by constipation (10%). Although QT interval prolongation of 2.4 ms was observed, no torsades de pointes were reported. Moreover, the survival analyses showed no increase in mortality in these patients. In conclusion, ranolazine is a safe, effective new drug for chronic stable angina; however, large-scale clinical trials are warranted to test various combination therapies.

POTASSIUM CHANNEL ACTIVATORS

Nicorandil is an arterial and venous dilator that improves coronary blood flow by opening the potassium channel, mimicking the natural process of ischaemic preconditioning. In comparison to placebo, nicorandil reduces death, nonfatal myocardial infarction, or unplanned hospitalisation in patients with chronic stable angina receiving other standard therapies. Recently, in a randomised trial comparing nicorandil and isosorbide mononitrate in patients with stable angina,[10] nicorandil (5 mg) improved exercise capacity and prevented angina attacks. In addition, nicorandil was as equally effective as nitrates in improving the time to 1 mm ST-depression, total exercise time and time to onset of chest pain. In this trial, the only adverse reaction observed in the nicorandil group was headache. Despite this favourable safety profile, previous studies have associated nicorandil with ulceration throughout the gastrointestinal tract,[11] however, the ulceration healed upon treatment withdrawal.

Table 1. Randomised, controlled trials assessing the safety and efficacy of ranolazine in patients with chronic stable angina.

Randomised trials	No. of patients	Clinical scenario	Intervention	Results
CARISA[6]	823	Chronic stable angina	Ranolazine (in patients already treated with atenolol, amlodipine, or diltiazem) versus placebo	Increase in exercise duration and time to angina. Decrease in the number of nitrate tablet consumption.
MARISA[7]	175*	Chronic stable angina	Ranolazine (used as antianginal monotherapy drug) versus placebo	Significant increase in exercise duration and time to 1 mm ST depression
ERICA[8]	564	Chronic stable angina	Ranolazine (in patients already treated with amlodipine) versus placebo	Significant decrease in frequency of angina episodes and glyceril trinitrate consumption

*This study initially included 191 patients, but efficacy data were available from only 175 patients.

ANGIOTENSIN-CONVERTING ENZYME INHIBITORS

Angiotensin-converting enzyme (ACE) inhibitors increase coronary blood flow by reversing angiotensin II-mediated vasoconstriction. Two ACE inhibitors have been tested in patients with refractory angina: enalapril and ramipril. Enalapril reduced exercise-induced myocardial ischaemia in normotensive patients with normal left ventricular function and angina refractory to β-blockade.[12] In the APRES trial,[13] ramipril reduced cardiac death, acute myocardial infarction, and heart failure in patients with stable angina and left ventricular dysfunction who underwent revascularisation. Although no evidence is available in no-option patients, most of these patients are treated with ACE inhibitors because of previous infarction, left ventricular dysfunction, or hypertension or because they are at high risk.

IVABRADINE

Ivabradine specifically inhibits the If sinoatrial pacemaker current, thereby reducing the heart rate both at rest and during exercise without any negative inotropic action or unmasked alpha-adrenergic coronary vasoconstriction. Initial clinical data have become recently available. Borer et al[14] demonstrated in a randomised study that ivabradine has beneficial effects on exercise tolerance in patients with stable angina, without a rebound effect after drug withdrawal. In addition, in two randomised trials, ivabradine showed comparable efficacy to amlodipine and atenolol in improving exercise tolerance.[15,16] In the recently published ASSOCIATE study,[17] patients with stable angina treated with atenolol were randomised to receive either ivabradine or placebo. At four months, patients treated with ivabradine showed a significant improvement in exercise capacity, and the drug was well tolerated. Therefore, ivabradine produced additional benefits in patients with persisting symptoms who were already being treated with atenolol. Although ivabradine may be an alternative treatment for no-option patients, its use requires further safety and efficacy assessments.

TESTOSTERONE

Intravenous[19] and transdermal administration[20] of testosterone has been shown to improve exercise-induced myocardial ischaemia in patients with stable angina, probably due to a coronary vasodilator effect and to increased blood haemoglobin levels. In 2008, Webb et al[21] published a randomised study comparing testosterone with placebo in patients with CAD who had low plasma levels of testosterone. Although no differences were seen in global myocardial perfusion as measured by magnetic resonance imaging (MRI), the authors reported a significant improvement in perfusion only in the myocardium supplied by unobstructed coronary arteries. Testosterone may have potential benefits in patients with myocardial ischaemia and low levels of testosterone; however, this approach cannot be recommended in all no-option patients.

INTRAVENOUS THROMBOLYTIC THERAPY, ADENOSINE, AND HEPARIN

Intermittent administration of low-dose thrombolytic therapy dissolves thrombus and improves coronary blood flow at both the epicardial and myocardial levels. In two trials of urokinase in patients with refractory angina pectoris,[22,23] exercise tolerance was increased as was time to ST-segment depression, without significant bleeding complications. However, both trials were limited by the small number of patients and the lack of a control group. Furthermore, urokinase is no longer available and other thrombolytic agents have not been studied for this purpose.

Adenosine may have a protective role, and heparin can accelerate the formation of coronary collaterals induced by ischaemia. Both drugs reduce the extent and severity of myocardial perfusion abnormalities in patients with refractory angina,[24] but larger studies are needed.

Invasive strategies for treatment of no-option patients

TRANSMYOCARDIAL LASER REVASCULARISATION

Transmyocardial laser revascularisation (TMLR) is a technique that uses laser ablation to create transmural channels in the ischaemic myocardium in order to restore myocardial perfusion. The physiologic premise behind the application of TMLR is based on the work of investigators who were seeking to emulate reptilian circulation in the mammalian heart by creating conduits for blood flow from the ventricular cavity into the myocardium.

Although the mechanism of action is unknown, several theories have been postulated. In one theory, confirmed by a 123-labeled meta-oidobenzylguanide scintigraphic study,[25] laser destruction of sympathetic nerve endings results in a form of cardiac denervation. Other theories include improvement in myocardial perfusion secondary to angiogenesis, or a placebo effect. Two types of laser have been used to treat refractory angina: the carbon dioxide (CO_2) laser system and the holmium:yttrium-aluminium-garnet (Ho:YAG) laser system. In animal studies, histologic effects have been similar after six weeks, but increased thermoacoustic damage has been seen with the YAG laser.

Several randomised trials evaluating TMLR as a sole therapy for no-option patients have shown symptomatic improvement with the use of TMLR as compared with medical treatment. The first study, conducted by Schofield et al,[26] involved 188 patients who were randomly assigned to either group. At one-year follow-up, improvement in angina scores and anti-anginal medications was observed in the TMLR group; however, exercise capacity did not improve. In a study by Frazier et al,[27] 192 patients were randomised to receive either TMLR or medical therapy. At 12-month follow-up, angina class, quality of life scores, and cardiac perfusion, as assessed by single-photon emission computed tomographic (SPECT) imaging, significantly improved. Similarly, Burkhoff et al[28] reported an increase in total exercise tolerance and a better quality of life at one year, but they found no differences in myocardial perfusion or ejection fraction between the two groups. Aaberge et al[29] described a significant reduction in angina symptoms and hospitalisations due to stable angina associated with TMLR, whereas left ventricular ejection fraction and mortality were seemingly unaffected. Interestingly, only one trial[30] has demonstrated survival benefits in patients randomised to TMLR; however, no improvement in myocardial perfusion was observed between the groups. Finally, a meta-analysis of seven randomised trials involving 1,053 patients that evaluated the effect of TMLR

showed a significant improvement in angina class but no improvement in survival.[31] The effect on long-term survival is a key component to establishing the risk/benefit profile of any treatment. In the above-mentioned trials, complications after TMLR were mainly cardiac related and included myocardial infarction, left ventricular failure, atrial fibrillation, and ventricular arrhythmias. Perioperative mortality ranged from 3-5% in most reports, but rates as high as 12% have been described.

TMLR has been used as an adjunctive therapy to CABG surgery. The safety and efficacy of CABG surgery combined with TMLR versus CABG surgery alone have been assessed in only two randomised trials in patients in whom complete revascularisation was not possible. Allen et al[32] randomised 263 patients to receive either combined therapy or CABG alone. At 1-year follow-up, survival benefits were observed in the combined group; however, the benefits were not associated with significant clinical improvement. In contrast, at 5-year follow-up, angina improvement was superior in the combined group, but no difference in survival was observed. Frazier et al[33] randomised 49 patients and described a significant reduction in recurrent angina at 4-year follow-up.

Percutaneous TMLR has been suggested in order to reduce perioperative mortality associated with surgical TMLR. Results of randomised unblinded studies using percutaneous TMLR are similar to those seen with open-chest TMLR—symptomatic improvement without an increase in the survival rate[34,35] or improvement in perfusion (thallium scintigraphy study) of the laser-treated regions.[36] However, two randomised and blinded trials failed to demonstrate any favourable effect, emphasising the importance of the placebo effect.[37,38]

In conclusion, TMLR studies have shown clinical improvement without an increase in the survival rate. Moreover, assessing the placebo effect is difficult, as symptomatic benefits do not always correlate with objective findings (improvement in the left ventricular ejection fraction or myocardial perfusion).[39,40] Therefore, TMLR should show a measurable physiologic benefit beyond the placebo effect before it can become an established therapeutic option for CAD.

ENHANCED EXTERNAL COUNTERPULSATION

Enhanced external counterpulsation (EECP) is a non-invasive procedure in which three sets of cuffs are used to compress the vascular beds of the leg and thigh in a sequential manner timed to the patient's electrocardiogram. The cuffs are wrapped around the patient's legs, and compressed air is used to apply sequential pressure (300 mmHg) from the lower legs to the lower and upper thighs during early diastole to propel blood back to the heart. Theoretically, this manoeuvre should result in a decrease in myocardial oxygen demand and an increase in coronary blood flow. This technique increases mean arterial blood pressure, retrograde aortic blood flow during diastole causing diastolic augmentation and increasing coronary perfusion along with venous return. In a multicentre, randomised controlled trial (MUST-EECP),[41] 139 patients with angina and documented ischaemia on treadmill exercise were randomised to receive 35 hours of active counterpulsation (300 mmHg of cuff pressure) or inactive counterpulsation (75 mmHg of cuff pressure) over a four- to seven-week period. The active counterpulsation group showed a significant decrease in angina episodes and nitroglycerin usage. Moreover, the time to ≥1 mm ST-segment depression increased in the active group as compared with the inactive group. Other registries with long-term follow-up that included more than 1,000 patients have shown similar results;[42,43] EECP produced a sustained improvement in angina and quality of life. However, these clinical effects have not been associated with consistent improvement in myocardial perfusion.[44]

The mechanisms of the sustained antianginal effect of EECP are debated. It has been suggested that the increased exercised capacity after EECP therapy may be attributed, in part, to a training effect. Other beneficial effects associated with EECP include a decrease in circulating levels of proinflammatory biomarkers[45] and an increase in the number and colony-forming capacity of circulating endothelial progenitor cells.[46] Most of the experience with EECP comes from uncontrolled studies; therefore, in the current guidelines for treating patients with chronic stable angina, EECP is proposed as an alternative treatment for patients with no options for standard procedures with a IIB level of recommendation.[47]

NEUROSTIMULATION

Two types of neurostimulation are used to palliate angina by interrupting or modulating the afferent neural signals through which pain is perceived: transcutaneous electrical nerve stimulation (TENS) and spinal cord stimulation (SCS).

TENS is based on the "gate-control theory": by stimulating large, non-nociceptive myelinated type A fibres transcutaneously at a high frequency, the system inhibits the impulse through smaller unmyelinated type C fibres, thereby reducing the activation of central pain receptors. In addition, TENS reduces sympathetic discharge, which leads to a decrease in cardiac work load and myocardial oxygen demand.[48] In studies of TENS, patients have shown an increase in exercise capacity with a reduction of ischaemia noted on exercise electrocardiogram, a decrease in symptoms of angina, and a reduction in nitrate use.[49] However, the development of skin irritations hampers the long-term use of the device.

In SCS, the epidural space is punctured at the level of the fourth or sixth thoracic vertebra, and an electrode is introduced in the T_1 to T_2 dorsal epidural space. An electrode stimulator is then placed subcutaneously in the upper left abdomen. Stimulation of these electrodes leads to a suppressed capacity of intrinsic cardiac sympathetic neurons to generate activity during myocardial ischaemia, thus decreasing pain sensation,[50] and to a redistribution of myocardial blood flow from non-ischaemic to ischaemic areas.[51] SCS does not appear to deprive the patient of a warning signal that leads to silent infarctions. Furthermore, SCS has anti-anginal and anti-ischaemic effects that seem to be secondary to a decrease in myocardial oxygen consumption. Three randomised trials comparing SCS with placebo have demonstrated a reduction in angina symptoms, an increase in exercise capacity, and a decrease in the degree of ST-segment depression at a given work load.[52-55] (Table 2) The Electrical Stimulation versus Coronary Bypass Surgery (ESBY) trial[56] showed that SCS and CABG surgery provide equal

Table 2. Randomised trials evaluating the safety and efficacy of spinal cord stimulation.

Randomised trials	No. of patients	Intervention	Results
Eddicks et al[52]	12	SCS versus placebo	Improvement in angina symptoms and walking distance
Hautvast et al[53]	25	SCS versus placebo	Improvement in exercise duration, time to angina, and ischaemic episodes on 48-hour ECG. Improvement in symptoms.
Jongste et al[54]	17	SCS versus placebo	Improvement in exercise duration, time to angina, and symptoms.
McNab et al[55]	30	SCS versus myocardial laser revascularisation	No difference in total exercise time and symptoms

SCS: spinal cord stimulation

symptom relief. However, the CABG group had better exercise capacity and less ST-segment depression on maximum and comparable workloads. Long term follow-up has shown no differences in 5-year mortality; therefore, SCS may be an option for patients with severe angina who have a high surgical risk.[57]

In summary, symptoms and ischaemia seem to improve with either TENS or SCS, although the data supporting SCS are more convincing. However, neither procedure affects survival, myocardial infarction, the need for repeat revascularisation, or left ventricular function. Concerns associated with this approach include the invasive nature of SCS, the cutaneous side effects of TENS, and the presence of a strong placebo effect.

GENE THERAPY

Gene transfer technology with the use of growth factors has been proposed to treat refractory angina by inducing angiogenesis and arteriogenesis. Several growth factors stimulate angiogenesis and arteriogenesis: vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF) and platelet derived growth factor (PDGF); platelet activating factor (PAF); angioproteins; cytokines, such as interleukin(IL)-6 and IL-8; master switch genes, hypoxia-inducible factor-1 alpha; and nitric oxide. Nevertheless, the most effective and safest delivery strategy for inducing angiogenesis in ischaemic myocardium has not been determined.

VEGF increases the rate of endothelial cell proliferation, thus improving myocardial perfusion and angina functional class. VEGF has been used intramuscularly as VEGF-encoding plasmids and with adenoviral vectors. All patients treated with VEGF reported improvement in symptoms with no evidence of systemic or cardiac specific toxicity in Phase I trials[58-60]. The VIVA (Vascular endothelial growth factor in ischaemia for Vascular Angiogenesis) trial, a double-blind, placebo-controlled trial, evaluated the safety and efficacy of intracoronary and intravenous infusions of recombinant human vascular endothelial growth factor protein (rhVEGF). The study comprised 178 patients with refractory angina who were randomised to receive low-dose rhVEGF, high-dose rhVEGF, or placebo. At 120-days, angina improved significantly only in patients treated with high-dose rhVEGF. No differences were reported among the groups in exercise treadmill test or myocardial perfusion. FGF increases the rate of endothelial cell proliferation. Patient perception of angina improved in preliminary studies in which basic FGF (bFGF) was administered in sustained-release microcapsules[61] and recombinant FGF (rFGF), delivered by the intracoronary or intravenous route, was reported to improve SPECT perfusion abnormalities.[62]

In the first randomised, double-blind, placebo-controlled trial of gene therapy, recombinant adenovirus 5 FGF-4 (AGENT trial[63] was delivered via the intracoronary route. Both the treatment and placebo groups showed improvement in exercise duration, thus demonstrating an important placebo effect. Only the subgroup of patients with exercise treadmill testing ≤ 10 minutes at baseline showed significant improvement in the treated group compared with the placebo group (1.6 vs 0.6 minutes, P=0.01, n=50). In the AGENT 2 trial[64] 52 patients with stable angina and reversible ischaemia were randomised to receive intracoronary injection of adenoviral particles containing a gene encoding fibroblast growth factor (Ad5FGF-4) or placebo. At eight weeks, Ad5FGF-4 injection had significantly reduced the size of the ischaemic defect (4.2% absolute, 21% relative; P <0.001), whereas placebo-treated patients showed no improvement (P=0.32). The AGENT 3 and 4 trials evaluated the efficacy of low- and high-dose treatment with Ad5FGF-4 in 532 patients with chronic angina in a randomised, double-blind, placebo-controlled fashion. Both studies were stopped because of a lack of efficacy after an interim analysis; however, a pooled data analysis from the two trials showed a significant improvement in total exercise treadmill testing time, time to 1 mm ST-segment depression, time to angina, and Canadian Cardiovascular Society class only in treated women, whereas there was no effect in men[65], suggesting a gender-specific beneficial effect of gene therapy. The randomised FIRST trial[66] evaluated the safety and efficacy of a single intracoronary infusion of recombinant FGF2 in 337 patients and found no improvement in exercise tolerance or myocardial perfusion. Although the use of gene therapy in patients with angina appears to be safe, efficacy data are controversial.

Another invasive technique that offers an alternative strategy for treating no-option patients is percutaneous *in situ* coronary venous arterialisation, but this approach is still in the experimental stage.

CELL THERAPY

Stem cell therapy has gained enormous interest since new insights have provided evidence that the heart may undergo a repair process in adulthood and that vasculogenesis may not be a paradigm found exclusively during embryonic development.[67,68] By promoting angiogenesis, stem cell therapy has the potential to improve anginal symptoms in patients with chronic CAD.

Mechanism of neovascularisation

The creation of new blood vessels involves three different processes of supplying blood flow in ischaemic tissues: angiogenesis, arteriogenesis and vasculogenesis. Arteriogenesis is the process in which a pre-existing arteriole of the resistance vessel class matures into an artery of the conductance vessel class, whereas angiogenesis is the formation of new vessels by sprouting of endothelial cells from pre-existing vessel capillaries that originate from a pre-existing capillary. Vasculogenesis refers to the *in situ* differentiation of endothelial precursor cells to form capillaries. Previous studies have shown that some bone marrow-derived endothelial progenitor cells in the peripheral circulation are able to form new vessels in the heart. Ashara et al[68] demonstrated that peripheral blood contains cells that can differentiate into endothelial cells *in vitro* and that endothelial progenitor cells are able to incorporate into sites of active angiogenesis *in vivo*. Using a canine bone marrow transplantation model in which the donor cells can be genetically recognised, Shi et al[69] observed that cells from the transplanted bone marrow can be mobilised to the peripheral circulation and can form an endothelial layer on a previously implanted Dacron graft. Quaini et al[70] used a cardiac chimerism model (female heart transplanted into a male host) to demonstrate that host cells can colonise the donor heart and can develop vessels.

Preclinical studies using a chronic ischaemia model

Very promising results have been obtained with proof-of-concept, pre-clinical experiments using bone marrow stem cells. A wide array of data has been generated that support the use of stem cells to repair cardiac tissue in diverse clinical scenarios. Bhakta et al,[71] using labelled cells and immunofluorescence microscopy, demonstrated that intracoronary delivered cells engrafted in the perivascular tissue. The safety of stem cell implantation has been shown in several studies. Li et al[72] found no significant differences in systemic biochemistry in a chronic ischaemic model. Furthermore, Goodchild et al[74] performed an electrophysiologic study seven weeks after stem cell treatment and reported no inducible ventricular arrhythmias in the treatment group, whereas arrhythmia was induced in one animal in the control group.

The efficacy of bone marrow-derived cells has been shown in several studies. Kinnaird[74] showed that cell transplantation enhanced recovery of collateral flow in a murine model of hind-limb ischaemia; perfusion as measured by laser Doppler perfusion imaging was significantly higher in the treatment group than in the control group. Similarly, Fuch et al[75] observed a significant improvement in myocardial perfusion in the ischaemic zone in the group treated with bone marrow-derived stem cells as compared with the control group in a pig model of chronic ischaemia. In addition, they observed an increase in myocardial contractility only in the treated pigs. Silva et al[76] described a significant increase in vascular density and a significant decrease in myocardial fibrosis in animals treated with allogeneic mesenchymal stem cells as compared with control animals. Kawamoto et al[77] demonstrated that NOGA- based transmyocardial injection of bone marrow-derived stem cell decreased the percentage of ischaemic area measure by NOGA and increased capillary density and left ventricular ejection fraction.

Clinical trials in no-option patients

Since 2003, several preliminary clinical studies have been performed to demonstrate the safety of bone marrow-derived stem cell implantation in no-option patients. Tse et al[78] prospectively studied eight patients with stable angina refractory to maximal medical therapy who were treated with catheter-based intramyocardial bone marrow transplantation. At 3-month follow-up, symptoms improved as did myocardial perfusion and target wall motion as assessed by MRI. In the same year, Perin et al[79] published a non-randomised, placebo-controlled study in which bone marrow cells were transplanted trans-endocardially in patients with severe heart failure due to ischaemic heart disease. The procedures were safe, and the authors reported a significant decrease in the reversible defect as measured with SPECT imaging and an improvement in myocardial volume oxygen consumption in the treated group as compared with the control group. Subsequently, three uncontrolled trials were published. Fuch et al[80] transplanted bone marrow mononuclear cells via direct NOGA-guided injections in 10 no-option patients (Figure 2). The treatment was safe and decreased the ischaemic burden as shown on

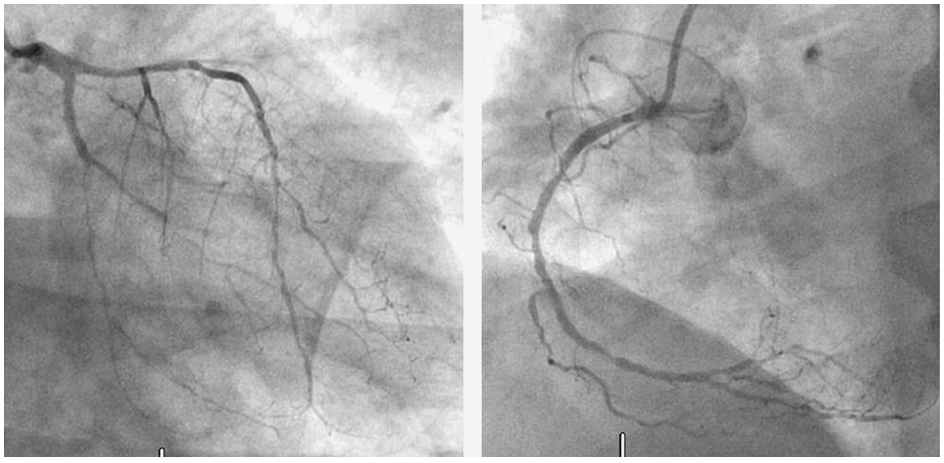

Figure 1. An example of a no-option patient. Left panel showing a severe and diffusely left anterior descending and distal circumflex and a sub-occluded marginal branch. Right panel diffuse disease in the right coronary artery

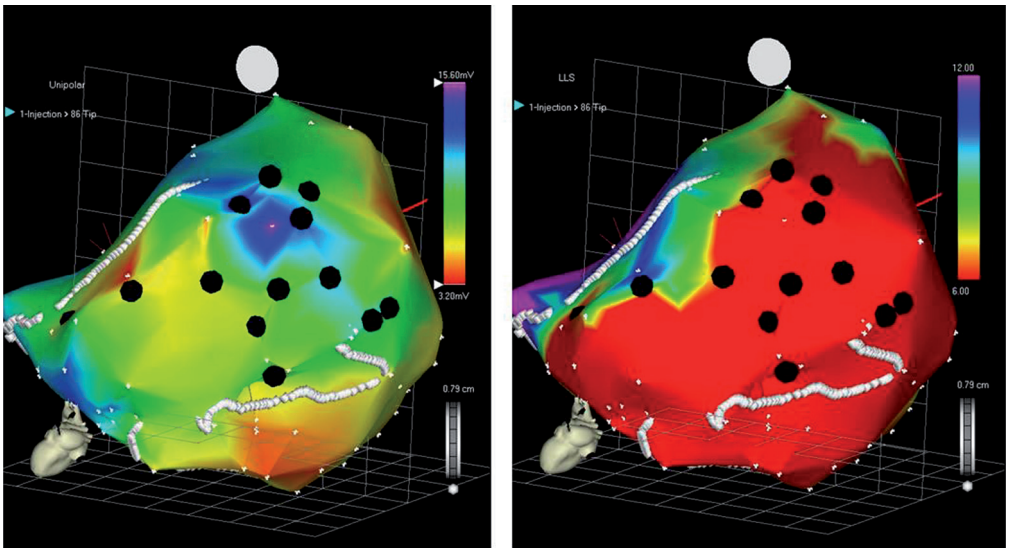

Figure 2. An example of a patient treated with stem cell with NOGA guided injections. Left panel univoltage map and right panel local shortening map. The black dots reflect the site of injections. In this particular case viable myocardium has been selected (high voltage and low local shortening)

perfusion imaging. Briguori et al[81] also treated 10 patients with NOGA-guided injections of bone marrow mononuclear cells and reported no cardiac events and clinical improvement at follow-up. In 20 patients, Beeres et al[82] showed significant improvement in left ventricular ejection fraction from 51% to 54% when compared with baseline (P<0.01) and a reduction in end-systolic volume from 97±50 to 88±42 ml (P<0.01) as measured by MRI.

The first randomised trial of a selected cell fraction derived from bone marrow was conducted by Losordo et al.[83] In this trial, 24 patients were randomly assigned to receive NOGA-guided injections of an enriched fraction of CD34+ cells or placebo in a dose-escalating study. Although this pilot study demonstrated the safety of the procedure, it was not powered to assess efficacy. The preliminary results of the FOCUS trial, a randomised, controlled trial assessing the safety and the efficacy of bone marrow mononuclear cells in no-option patients, were recently presented by Perin et al (personal communication). At three months, they found significant improvement in both the Canadian Cardiovascular Society class and the percentage of reversible ischaemia as measured by SPECT imaging in the treatment group when compared with the control group. To date, stem cell studies have certainly only demonstrated the safety of this treatment in this clinical scenario; nevertheless, further clinical trials are warranted to establish the efficacy of this promising field.

References

1. Mukherjee D, Bhatt DL, Roe MT, Patel V, Ellis SG. Direct myocardial revascularization and angiogenesis --how many patients might be eligible? *Am J Cardiol.* 1999;84:598-600.

2. Mukherjee D, Bhatt DL, Roe MT, Patel V, Ellis SG. Direct myocardial revascularization and angiogenesis--how many patients might be eligible? *Am J Cardiol.* 1999 1;84:598-600, A8.

3. Weintraub WS, Jones EL, Craver JM, Guyton RA. Frequency of repeat coronary bypass or coronary angioplasty after coronary artery bypass surgery using saphenous venous grafts. *Am J Cardiol.* 1994;73:103-12.

4. Marzilli M, Klein WW. Efficacy and tolerability of trimetazidine in stable angina: a meta-analysis of randomized, double-blind, controlled trials. *Coron Artery Dis* 2003;14:171-9.

5. Antzelevitch C, Belardinelli L, Wu L, Fraser H, Zygmunt AC, Burashnikov A, Diego JM, Fish JM, Cordeiro JM, Goodrow RJ Jr, Scornik F, Perez G. Electrophysiologic properties and antiarrhythmic actions of a novel antianginal agent. *J Cardiovasc Pharmacol Ther.* 2004;9 Suppl 1:S65-83.

6. Chaitman BR, Pepine CJ, Parker JO, Skopal J, Chumakova G, Kuch J, Wang W,Skettino SL, Wolff AA; Combination Assessment of Ranolazine In Stable Angina(CARISA) Investigators. Effects of ranolazine with atenolol, amlodipine, or diltiazem on exercise tolerance and angina frequency in patients with severe chronic angina: arandomized controlled trial. *JAMA.* 2004;291:309-16.

7. Chaitman BR, Skettino SL, Parker JO, Hanley P, Meluzin J, Kuch J, Pepine CJ, Wang, Nelson JJ, Hebert DA, Wolff AA; MARISA Investigators. Anti-ischemic effects and long-term survival during ranolazine monotherapy in patients with chronic severe angina. *J Am Coll Cardiol.* 2004; 43:1375-82.

8. Stone PH, Gratsiansky NA, Blokhin A, Huang IZ, Meng L; ERICA Investigators. Antianginal efficacy of ranolazine when added to treatment with amlodipine: the ERICA (Efficacy of Ranolazine in Chronic Angina) trial. *J Am Coll Cardiol.* 2006 1;48:566-75.

9. Koren MJ, Crager MR, Sweeney M. Long-term safety of a novel antianginal agent in patients with severe chronic stable angina: the Ranolazine Open Label Experience (ROLE). *J Am Coll Cardiol.* 2007;49:1027-34.

10. Zhu WL, Shan YD, Guo JX, Wei JP, Yang XC, Li TD, Jia SQ, He Q, Chen JZ, Wu ZG, Li ZQ, You K. Double-blind, multicenter, active-controlled, randomized clinical trial to assess the safety and efficacy of orally administered nicorandil in patients with stable angina pectoris in China. *Circ J.* 2007;71:826-33.

11. Nicorandil: serious gastrointestinal ulceration. Prescrire Int. 2008 Jun;17(95):110-1.

12. van den Heuvel AF, Dunselman PH, Kingma T, Verhorst P, Boomsma F, van Gilst WH, van Veldhuisen DJ. Reduction of exercise-

induced myocardial ischemia during add-on treatment with the angiotensin-converting enzyme inhibitor enalapril in patients with normal left ventricular function and optimal beta blockade. *J Am Coll Cardiol* 2001;37:470-4.

13. Kjoller-Hansen L, Steffensen R, Grande P. The Angiotensin-converting Enzyme Inhibition Post Revascularization Study (APRES). *J Am Coll Cardiol* 2000;35:881-8.

14. Borer JS, Fox K, Jaillon P, Lerebours G; Ivabradine Investigators Group. Antianginal and antiischemic effects of ivabradine, an I(f) inhibitor, in stable angina: a randomized, double-blind, multicentered, placebo-controlled trial. *Circulation* 2003;107:817-23.

15. Tardif JC, Ford I, Tendera M, Bourassa MG, Fox K. Efficacy of ivabradine, a new selective If inhibitor, compared with atenolol in patients with chronic stable angina. *Eur Heart J* 2005;26:2529-36.

16. Ruzyllo W, Tendera M, Ford I, Fox KM. Antianginal efficacy and safety of ivabradine compared with amlodipine in patients with stable effort angina pectoris: a 3-month randomised, double-blind, multicentre, noninferiority trial. *Drugs.* 2007;67:393-405.

17. Tardif JC, Ponikowski P, Kahan T; for the ASSOCIATE study investigators. Efficacy of the If current inhibitor ivabradine in patients with chronic stable angina receiving beta-blocker therapy: a 4 month, randomized, placebo-controlled trial. *Eur Heart J.* 2009 Jan 9.

18. English KM, Steeds RP, Jones TH, Diver MJ, Channer KS. Low-dose transdermal testosterone therapy improves angina threshold in men with chronic stable angina: A randomized, double-blind, placebo-controlled study. *Circulation* 2000;102:1906-11.

19. Rosano GM, Leonardo F, Pagnotta P, Pelliccia F, Panina G, Cerquetani E, della Monica PL, Bonfigli B, Volpe M, Chierchia SL. Acute anti-ischemic effect of testosterone in men with coronary artery disease. *Circulation* 1999;99:1666-70.

20. English KM, Steeds RP, Jones TH, Diver MJ, Channer KS. Low-dose transdermal testosterone therapy improves angina threshold in men with chronic stable angina: A randomized, double-blind, placebo-controlled study. *Circulation.* 2000;102:1906-11

21. Webb CM, Elkington AG, Kraidly MM, Keenan N, Pennell DJ, Collins P. Effects of oral testosterone treatment on myocardial perfusion and vascularfunction in men with low plasma testosterone and oronary heart disease. *Am J Cardiol.* 2008;101:618-24.

22. Schoebel FC, Leschke M, Jax TW, Stein D, Strauer BE. Chronic intermittent urokinase therapy in patients with end-stage coronary artery disease and refractory angina pectoris: a pilot study. *Clin Cardiol* 1996;19:115-20.

23. Leschke M, Schoebel FC, Mecklenbeck W, Stein D, Jax TW, Müller-Gärtner HW, Strauer BE. Long-term intermittent urokinase therapy in patients with end-stage coronary artery disease and refractory angina pectoris: a randomized dose-response trial. *J Am Coll Cardiol* 1996;27:575-84.

24. Barron HV, Sciammarella MG, Lenihan K, Michaels AD, Botvinick EH. Effects of the repeated administration of adenosine and heparin on myocardial perfusion in patients with chronic stable angina pectoris. *Am J Cardiol* 2000;85:1-7.

25. Beek JF, van der Sloot JA, Huikeshoven M, Verberne HJ, van Eck-Smit BL, van der Meulen J, Tijssen JG, van Gemert MJ, Tukkie R. Cardiac denervation after clinical transmyocardial laser revascularization: short-term and long-term iodine 123-labeled meta-iodobenzylguanide scintigraphic evidence. *J Thorac Cardiovasc Surg.* 2004;127:517-24

26. Schofield PM, Sharples LD, Caine N, Burns S, Tait S, Wistow T, Buxton M, Wallwork J. Transmyocardial laser revascularisation in patients with refractory angina: a randomised controlled trial. *Lancet.* 1999;353:519-24.

27. Frazier OH, March RJ, Horvath KA. Transmyocardial revascularization with a carbon dioxide laser in patients with end-stage coronary artery disease. *N Engl J Med.* 1999;341:1021-8.

28. Burkhoff D, Schmidt S, Schulman SP, Myers J, Resar J, Becker LC, Weiss J, Jones JW. Transmyocardial laser revascularisation compared with continued medical therapy for treatment of refractory angina pectoris: a prospective randomised trial. ATLANTIC Investigators. Angina Treatments-Lasers and Normal Therapies in Comparison. *Lancet.* 1999;354:885-90.

29. Aaberge L, Rootwelt K, Blomhoff S, Saatvedt K, Abdelnoor M, Forfang K. Continued symptomatic improvement three to five years after transmyocardial revascularization with CO(2) laser: a late clinical follow-up of the Norwegian Randomized trial with transmyocardial revascularization. *J Am Coll Cardiol.* 2002 15;39:1588-93.

30. Allen KB, Dowling RD, Angell WW, Gangahar DM, Fudge TL, Richenbacher W, Selinger SL, Petracek MR, Murphy D. Transmyocardial revascularization: 5-year follow-up of a prospective, randomized multicenter trial. *Ann Thorac Surg.* 2004 ;77:1228-34.

31. Liao L, Sarria-Santamera A, Matchar DB, Huntington A, Lin S, Whellan DJ, Kong DF. Meta-analysis of survival and relief of angina pectoris after transmyocardial revascularization. *Am J Cardiol.* 2005;95:1243-5.

32. Allen KB, Dowling RD, DelRossi AJ, Realyvasques F, Lefrak EA, Pfeffer TA,Fudge TL, Mostovych M, Schuch D, Szentpetery S, Shaar CJ. Transmyocardial laser revascularization combined with coronary artery bypass grafting: a multicenter, blinded, prospective, randomized, controlled trial. *J Thorac Cardiovasc Surg.* 2000 ;119:540-9

33. Frazier OH, March RJ, Horvath KA. Transmyocardial revascularization with a carbon dioxide laser in patients withend-stage coronary artery disease. *N Engl J Med.* 1999 30;341:1021-8.

34. Oesterle SN, Sanborn TA, Ali N, Resar J, Ramee SR, Heuser R, Dean L, Knopf W, Schofield P, Schaer GL, Reeder G, Masden R, Yeung AC, Burkhoff D. Percutaneous transmyocardial laser revascularisation for severe angina: the PACIFIC randomised trial. Potential Class Improvement From Intramyocardial Channels. *Lancet.* 2000 18;356:1705-10.

35. Whitlow PL, DeMaio SJ Jr, Perin EC, O'Neill WW, Lasala JM, Schneider JE, McKeever LS, Ezratty AM, Knopf WD, Powers ER, Shawl FA; Eclipse Investigators. One-year results of percutaneous myocardial revascularization for refractory angina pectoris, *Am J Cardiol* 2003;91: 1342–1346.

36. Lauer B, Junghans U, Stahl F, Kluge R, Oesterle SN, Schuler G. Catheter-based percutaneous myocardial laser revascularization in patients with end-stage coronary artery disease. *J Am Coll Cardiol.* 1999;34:1663-70.

37. Stone GW, Teirstein PS, Rubenstein R, Schmidt D, Whitlow PL, Kosinski EJ, Mishkel G, Power JA. A prospective, multicenter, randomized trial of percutaneous transmyocardial laser revascularization in patients with nonrecanalizable chronic total occlusions. *J Am Coll Cardiol.* 2002;39:1581-7.

38. Leon MB, Kornowski R, Downey WE, Weisz G, Baim DS, Bonow RO, Hendel RC, Cohen DJ, Gervino E, Laham R, Lembo NJ, Moses JW, Kuntz RE. A blinded, randomized, placebo-controlled trial of percutaneous laser myocardial revascularization to improve angina symptoms in patients with severe coronary disease. *J Am Coll Cardiol.* 2005;46:1812-9.

39. Rimoldi O, Burns SM, Rosen SD, Wistow TE, Schofield PM, Taylor G, Camici PG. Measurement of myocardial blood flow with positron emission tomography before and after transmyocardial laser revascularization. *Circulation.* 1999;100(19 Suppl):II134-8

40. Kavanagh GJ, Whittaker P, Prejean CA Jr, Firth BR, Kloner RA, Kay GL. Dissociation between improvement in angina pectoris and myocardial perfusionafter transmyocardial revascularization with an excimer laser. *Am J Cardiol.* 2001;87:229-31, A9

41. Arora RR, Chou TM, Jain D, Fleishman B, Crawford L, McKiernan T, Nesto RW. The multicenter study of enhanced external counterpulsation (MUST-EECP): effect of EECP on exercise-induced myocardial ischemia and anginal episodes. *J Am Coll Cardiol.*1999;33:1833-40.

42. Lawson WE, Hui JC, Kennard ED, Kelsey SF, Michaels AD, Soran O. International Enhanced External Counterpulsation Patient Registry Investigators. Two-year outcomes in patients with mild refractory angina treated with enhanced external counterpulsation. *Clin Cardiol.* 2006;29:69-73.

43. Loh PH, Cleland JG, Louis AA, Kennard ED, Cook JF, Caplin JL, Barsness GW, Lawson WE, Soran OZ, Michaels AD. Enhanced external counterpulsation in the treatment of chronic refractory angina:a long-term follow-up outcome from the International Enhanced External Counterpulsation Patient Registry. *Clin Cardiol.* 2008 31:159-64.

44. Michaels AD, Raisinghani A, Soran O, de Lame PA, Lemaire ML, Kligfield P, Watson DD, Conti CR, Beller G. The effects of enhanced external counterpulsation on myocardial perfusion in patients with stable angina: a multicenter radionuclide study. *Am Heart J.* 2005;150:1066-73.

45. Casey DP, Conti CR, Nichols WW, Choi CY, Khuddus MA, Braith RW. Effect of enhanced external counterpulsation on inflammatory cytokines and adhesion molecules in patients with angina pectoris and angiographic coronary artery disease. *Am J Cardiol.* 2008;101:300-2.

46. Barsheshet A, Hod H, Shechter M, Sharabani-Yosef O, Rosenthal E, Barbash IM, Matetzky S, Tal R, Bentancur AG, Sela BA, Nagler A, Leor J. The effects of external counter pulsation therapy on circulating endothelial progenitor cells in patients with angina pectoris. *Cardiology* 2008;110:160-6.

47. Gibbons RJ, Abrams J, Chatterjee K, Daley J, Deedwania PC, Douglas JS, Ferguson TB Jr, Fihn SD, Fraker TD Jr, Gardin JM, O'Rourke RA, Pasternak RC, Williams SV, Gibbons RJ, Alpert JS, Antman EM, Hiratzka LF, Fuster V, Faxon DP, Gregoratos G, Jacobs AK, Smith SC Jr; American College of Cardiology; American Heart Association Task Force on Practice Guidelines. Committee on the Management of Patients With Chronic Stable Angina. ACC/AHA 2002 guideline update for the management of patients with chronic stable angina--summary article: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines (Committee on the Management of Patients With Chronic Stable Angina. *Circulation.* 2003 ;107:149-58.

48. Mannheimer C, Carlsson CA, Emanuelsson H, Vedin A, Waagstein F, Wilhelmsson C. The effects of transcutaneous electrical stimulation in patients with severe angina pectoris. *Circulation.* 1985;71:308-16.

49. Hautvast RWM, Brouwer J, DeJonste MJL, Lie KI. Effect of spinal cord stimulation on heart rate variability and myocardial ischemia in patients with chronic intractable angina pectoris: a prospective ambulatory electrocardiographic study. *Clin Cardiol.* 1998;21:33-8.

50. Foreman RD, Linderoth B, Ardell JL, Barron KW, Chandler MJ, Hull SS Jr, TerHorst GJ, DeJongste MJ, Armour JA. Modulation of intrinsic cardiac neurons by spinal cord stimulation: implications for its therapeutic use in angina pectoris. *Cardiovasc Res.* 2000;47:367-75.

51. Murray S, Collins PD, James MA. Neurostimulation treatment for angina pectoris. *Heart.* 2000;83:217-20.

52. Eddicks S, Maier-Hauff K, Schenk M, Müller A, Baumann G, Theres H. Thoracic spinal cord stimulation improves functional status and relieves symptom in patients with refractory angina pectoris: the first placebo-controlled randomised study. *Heart.* 2007;93:585-90.

53. Hautvast RW, DeJongste MJ, Staal MJ, van Gilst WH, Lie KI. Spinal cord stimulation in chronic intractable angina pectoris: a randomized, controlled efficacy study. *Am Heart J.* 1998;136:1114-20.

54. de Jongste MJ, Hautvast RW, Hillege HL, Lie KI. Efficacy of spinal cord stimulation as adjuvant therapy for intractable angina pectoris: a prospective, randomized clinical study. Working Group on Neurocardiology. *J Am Coll Cardiol.* 1994;23:1592-7.

55. McNab D, Khan SN, Sharples LD, Ryan JY, Freeman C, Caine N, Tait S, Hardy I, Schofield PM. An open label, single-centre, randomized trial of spinal cord stimulation vs. percutaneous myocardial laser revascularization in patients with refractory angina pectoris: the SPiRiT trial. *Eur Heart J.* 2006;27:1048-53.

56. Mannheimer C, Eliasson T, Augustinsson LE, Blomstrand C, Emanuelsson H, Larsson S, Norrsell H, Hjalmarsson A. Electrical stimulation versus coronary artery bypass surgery in severe angina pectoris: the ESBY study. *Circulation.* 1998;37:1157-63.

57. Ekre O, Eliasson T, Norrsell H, Währborg P, Mannheimer C; Electrical Stimulation versus Coronary Artery Bypass Surgery in Severe Angina Pectoris. Long-term effects of spinal cord stimulation and coronary artery bypass grafting on quality of life and survival in the ESBY study. *Eur Heart J.* 2002 ;23:1938-45.

58. Baumgartner I, Pieczek A, Manor O, Blair R, Kearney M, Walsh K, Isner JM. Constitutive expression of ph VEGF 165 after intramuscular gene transfer promotes collateral vessel development in patients with critical limb ischemia. *Circulation.* 1998;97:1114-23.

59. Losordo DW, Vale PR, Symes JF, Dunnington CH, Esakof DD, Maysky M, Ashare AB, Lathi K, Isner JM. Gene therapy for myocardial angiogenesis: initial clinical results with direct myocardial injection of ph VEGF 165 as sole therapy for myocardial ischemia. *Circulation.* 1998;98:2800-4.

60. 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.

61. 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, placebocontrolled trial. *Circulation.* 1999;100: 1865-71.

62. 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 patients with stable angina pectoris. *Circulation* 2002;102:1605-10.

63. 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 in patients with stable angina pectoris. *Circulation.* 2002;105:1291.

64. Grines CL, Watkins MW, Mahmarian JJ, Iskandrian AE, Rade JJ, Marrott P, Pratt C, Kleiman N; Angiogene GENe Therapy (AGENT-2) Study Group. 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;42:1339-47.

65. Henry TD, Grines CL, Watkins MW, Dib N, Barbeau G, Moreadith R, Andrasfay T,Engler RL. Effects of Ad5FGF-4 in patients with angina: an analysis of pooled data from the AGENT-3 and AGENT-4 trials. *J Am Coll Cardiol.* 2007;50:1038-46.

66. 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.

67. 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; 103:2776-2779.

68. Asahara T, Murohara T, Sullivan A, Silver M, van der Zee R, Li T, Witzenbichler B, Schatteman G, Isner JM. Isolation of putative progenitor endothelial cells for angiogenesis. *Science.* 1997;275:964-7.

69. 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.

70. Quaini F, Urbanek K, Beltrami AP, Finato N, Beltrami CA, Nadal-Ginard B, Kajstura J, Leri A, Anversa P. Chimerism of the transplanted heart. *N Engl J Med.* 2002 ;346:5-15.

71. Bhakta S, Greco NJ, Finney MR, Scheid PE, Hoffman RD, Joseph ME, Banks JJ,Laughlin MJ, Pompili VJ. The safety of autologous intracoronary stem cell injections in a porcine model of chronic myocardial ischemia. *J Invasive Cardiol.* 2006;18:212-8.

72. Li TS, Hamano K, Hirata K, Kobayashi T, Nishida M. The safety and feasibility of the local implantation of autologous bone marrow cells for ischemic heart disease. *J Card Surg.* 2003;18 Suppl 2:S69-75

73. Goodchild T, Pang W, Tondato F, Cui J, Otsuka Y, Frowein S, Ungs M, Robinson K, Poznansky M, Chronos N. Safety of intramyocardial injection of autologous bone marrow cells to treatmyocardial ischemia in pigs. *Cardiovasc Revasc Med.* 2006;7(3):136-45.

74. Kinnaird T, Stabile E, Burnett MS, Epstein SE. Bone-marrow-derived cells for enhancing collateral development: mechanisms, animal data, and initial clinical experiences. *Circ Res.* 2004;95:354-63.

75. Fuchs S, Baffour R, Zhou YF, Shou M, Pierre A, Tio FO, Weissman NJ, Leon MB, Epstein SE, Kornowski R. Transendocardial delivery of autologous bone marrow enhances collateral perfusion and regional function in pigs with chronic experimental myocardial ischemia. *J Am Coll Cardiol.* 2001;37:1726-32.

76. Perin EC, Silva GV, Assad JA, Vela D, Buja LM, Sousa AL, Litovsky S, Lin J, Vaughn WK, Coulter S, Fernandes MR, Willerson JT. Comparison of intracoronary and transendocardial delivery of allogeneic mesenchymal cells in a canine model of acute myocardial infarction. *J Mol Cell Cardiol.* 2008;44:486-95.

77. Kawamoto A, Tkebuchava T, Yamaguchi J, Nishimura H, Yoon YS, Milliken C, UchidaS, Masuo O, Iwaguro H, Ma H, Hanley A, Silver M, Kearney M, Losordo DW, Isner JM, Asahara T. Intramyocardial transplantation of autologous endothelial progenitor cells for therapeutic neovascularization of myocardial ischemia. *Circulation.* 2003;107:461-8.

78. 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.

79. Perin EC, Dohmann HF, Borojevic R, Silva SA, Sousa AL, Mesquita CT, Rossi MI, Carvalho AC, Dutra HS, Dohmann HJ, Silva GV, Belém 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.

80. 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.

81. Briguori C, Reimers B, Sarais C, Napodano M, Pascotto P, Azzarello G, Bregni M, Porcellini A, Vinante O, Zanco P, Peschle C, Condorelli G, Colombo A. Direct intramyocardial percutaneous delivery of autologous bone marrow in patients with refractory myocardial angina. *Am Heart J.* 2006;151:674-80.

82. Beeres SL, Bax JJ, Kaandorp TA, Zeppenfeld K, Lamb HJ, Dibbets-Schneider P, Stokkel MP, Fibbe WE, de Roos A, van der Wall EE, Schalij MJ, Atsma DE. Usefulness of intramyocardial injection of autologous bone marrow-derived mononuclear cells in patients with severe angina pectoris and stress-induced myocardial ischemia. *Am J Cardiol.* 2006;97:1326-31.

83. 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.