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# Autologous Bone Marrow–Derived Stem Cell Therapy in Combination with TMLR. A Novel Therapeutic Option for Endstage Coronary Heart Disease: Report on 2 Cases

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## ABSTRACT

We report 2 cases in which patients with coronary heart disease not amenable for conventional revascularization underwent transmyocardial laser revascularization (TMLR) and implantation of AC133$^+$ bone-marrow stem cells. The reason for using TMLR in combination with stem cell application is to take advantage of the synergistic angiogenic effect. The local inflammatory reaction induced by TMLR should serve as an informational platform for stem cells and may trigger their angiogenic differentiation. Functional analysis of myocardial performance after treatment in these 2 cases revealed dramatic improvement of the wall motion at the site of the TMLR and stem cell application. Because TMLR does not enhance myocardial contractility and there was no angiographic evidence of major collaterals to the ischemic region in either patient, we assume that the synergistic effect of stem cells and TMLR-induced angiogenesis occurred; however, our assumption is of a speculative nature. We think that TMLR in combination with stem cell transplantation might become a novel revascularization therapy for ischemic myocardium.

## INTRODUCTION

Up to 15% of patients with end-stage coronary artery disease suffer from disabling anginal symptoms despite maximal pharmacotherapy and conventional revascularizations [Wilke 2000]. Transmyocardial laser revascularization (TMLR) is currently approved by the US Food and Drug Administration for patients with disabling angina for which blockages are too diffuse to be treated with a bypass graft or angioplasty alone and for patients with microvascular disease [Allen 1999, Frazier 1999, Wilke 2000]. Recent clinical and experimental trials have shown that symptomatic improvement after TMLR in patients with refractory angina is probably related to neoangiogenesis [Karabatsch 1996].

Implantation of bone marrow stem cells in the heart is reported to be a possible method for myocardial regeneration after myocardial infarction [Orlic 2001]. The ischemic myocardium at the border of the infarct zone or in the hibernating myocardium can be rescued by 2 mechanisms, angiogenesis and myogenesis. The transplantation of different cell populations such as CD34$^+$ cells, c-kit$^+$Lin$^-$ cells, or myoblasts has been shown to improve cardiac function in animal studies [Kocher 2001, Menasche 2001, Orlic 2001].

A population of mononuclear cells (MNCs), the AC133$^+$ stem cells, has strong angiogenic potential [Quirici 2001]. Recent data also show the ability of AC133$^+$ cells to differentiate into myoendothelial cell lines in vitro and in vivo [Pesce 2003].

Significant improvement of cardiac function in human postinfarction myocardium has been shown to occur after transplantation of AC133$^+$ cells [Stamm 2003]. The transplantation of MNCs also improves cardiac function [Strauer 2002], but when the whole MNC population is transplanted, a much higher number of cells must be injected to achieve a comparable effect because the MNCs are likely to consist of only less than 2% AC133$^+$ cells [Strauer 2002].

The theoretical reasoning behind the use of TMLR in combination with stem cell application is the synergistic angiogenic effect. Local inflammatory reaction induced by TMLR should serve as an informational platform for stem cells and may trigger their angiogenic differentiation [Karabatsch 1996].

## CASE REPORT

Two patients with coronary heart disease not amenable to conventional revascularization underwent implantation with AC133$^+$ cells. The region of the heart chosen for injection was predefined preoperatively based on data from coronary angiography, single-photon emission computed tomography, and magnetic resonance imaging (MRI) that indicated nonsuitablity for bypass grafting and evidence of reversible

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ischemia. Exclusion criteria included abnormal hemoglobin, platelet count and function, or leukocyte count or conditions that may adversely affect bone marrow, such as malignancy or human immunodeficiency virus infection. Informed consent was obtained from both patients.

Patient bone marrow was aspirated after the induction of anesthesia; heparin-coated syringes were used to obtain 200 to 240 mL of bone marrow aspirate from the iliac crest. The aspirate was collected in blood bags and washed with EDTA/phosphate buffered saline/25 mL 20% human albumin. The cell suspension was filtered to remove bone spicula and then processed by a GMP-certified cell selection unit (Clinimac; Miltenyi Biotec, Cologne, Germany) to select for $AC133^+$ cells. After approximately 160 minutes, the enriched MNCs were ready for intramyocardial injection.

During cell preparation extracorporal circulation (ECC) was established and coronary bypass grafting done. During ECC, 17 to 20 laser channels were shot using a $CO_2$ laser (PLC Medical Systems, Franklin, MA, USA) in 1 of 3 predefined and nonrevascularized regions, anterior, lateral, and posterior wall. The minimum distance between the laser channels was 1 cm. Prepared stem cells were then injected around the laser channels according to the protocol, approximately 500.000 $ACC133^+$ cells per channel.

Aliquots from the bone marrow aspirate and the injected cell fraction were collected. The number of MNCs was registered by a cell counter (Sysmex, Mundelein, IL, USA). Aliquots were analyzed by fluorescence-activated cell sorting using anti-AC133, anti-CD34, anti-CD45, and propidium iodide (Miltenyi Biotec).

To assess the global and segmental left ventricular performance, transthoracic echocardiography (TTE) and cardiac MRI were done prior to and after surgery.

## RESULTS

Two patients, aged 69 and 57 years, underwent a surgical procedure combining TMLR with stem cell application according to the following protocol:

### *Patient 1*

We performed coronary bypass grafts to the first marginal artery and the descending posterior artery. The nonrevascularized anterior wall was treated by TMLR in combination with bone marrow cell (BMC) application; 200 mL of bone marrow aspirate was taken, and $ACC133^+$ cells were prepared for application after 160 minutes. The filtered bone marrow had normal morphology, and the microbiological screening showed no contamination. During surgery 19 channels were done, and $7.5 \times 10^6$ $ACC133^+$ stem cells with a purity of 97% were applied, distributed equally around the laser channels.

TTE performed 3 months after the operation showed an improvement of ejection fraction (preoperative 15% versus postoperative 37%) and enhanced anterior wall contraction performance. On the third postoperative month cardiac MRI showed no evidence of myocardial edema on T2-weighted sequences. Electrocardiogram (ECG)-triggered bright-blood sequences in long-axis, short-axis (Figure), and 4-chamber views showed a significant improvement of wall motion in the anterolateral free wall, the septum, and the posterolateral wall. The end systolic wall thickness in the anterolateral wall increased from 6 mm to 8 mm and from 5 mm to 12 mm (see Figure). Left ventricular ejection fraction increased from 25% to 32%.

### *Patient 2*

The coronary bypass grafts were done to the second marginal and the descending posterior artery. The nonrevascularized anterior wall was treated by TMLR in combination with BMC application; 240 mL of bone marrow aspirate was taken, and $ACC133^+$ cells were prepared for application after 160 minutes. During surgery 17 channels were done, and $9.18 \times 10^6$ $ACC133^+$ stem cells with a purity of 90% were applied, distributed equally around the laser channels.

TTE performed 3 months after the operation showed an improvement of ejection fraction (preoperative 24% versus postoperative 47%) and enhanced anterior wall contraction performance. Cardiac MRI on the third postoperative month showed no evidence of myocardial edema on T2-weighted sequences. ECG-triggered bright-blood sequences in the long-axis, short-axis, and 4-chamber view showed a significant improvement of wall motion in the anterolateral free wall and the posterolateral wall (data not shown). Left ventricular ejection fraction increased from 24% to 46%.

Beginning on the second postoperative day, both patients had postprocedure supraventricular arrhythmia, which was treated successfully with beta-blockers. The mean time for both operative procedures was 3 hours 30 minutes.

## DISCUSSION

For patients with coronary heart disease not amenable to interventional or surgical treatment, different therapeutic strategies have been developed; however, stem cell therapy seems to be one of the most promising therapeutic options [Strauer 2002, Stamm 2003].

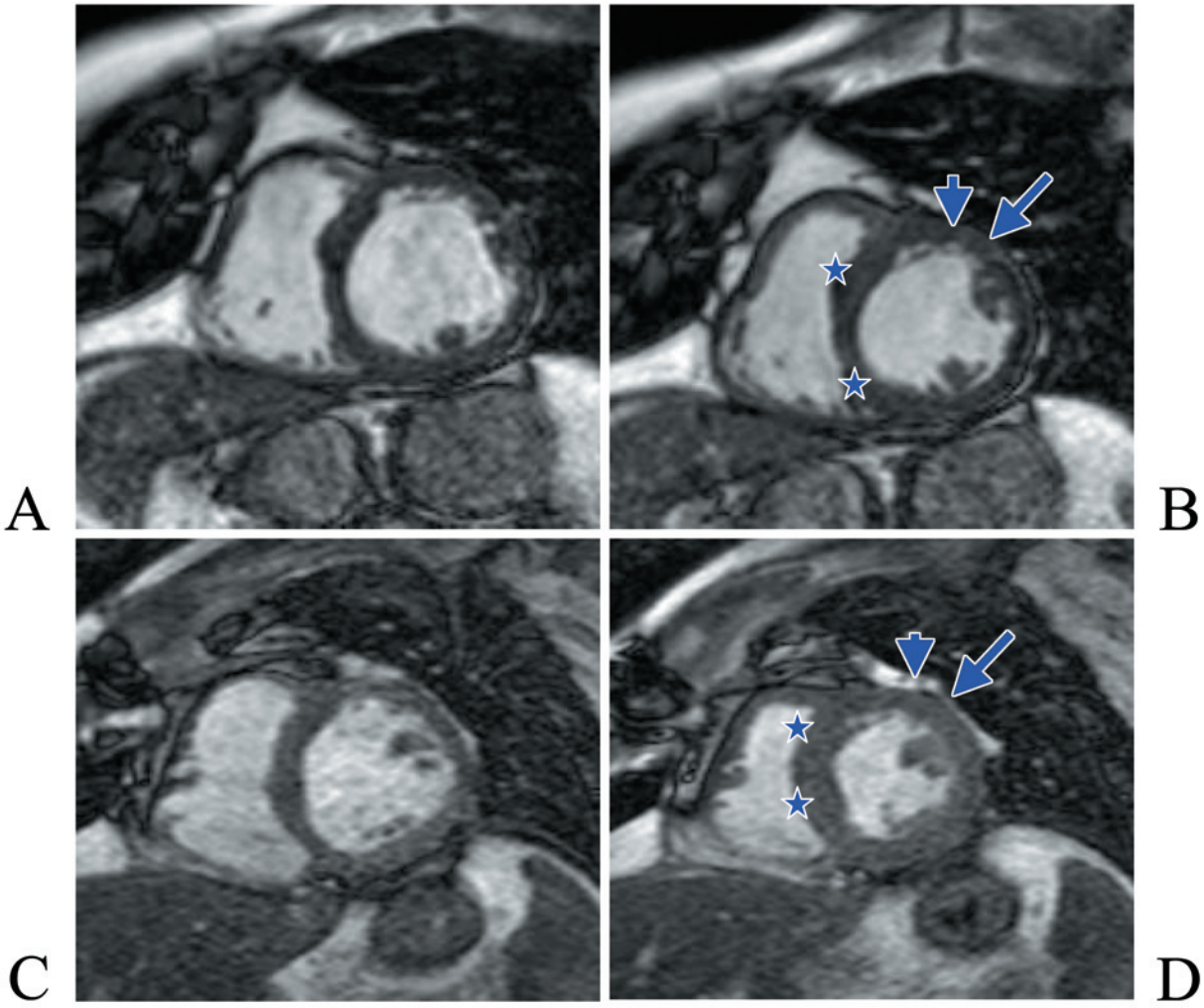
Early experimental studies have shown that transplantation of autologous BMCs in murine ischemic myocardium reduces the scar area [Kocher 2001, Orlic 2001].

Anversa and associates [Orlic 2001] could demonstrate that $c\text{-}kit^+Lin^-$ stem cells transplanted into postinfarction myocardium can differentiate into cardiomyocytes, and Hakuno et al [1999] induced the differentiation of mesenchymal BMCs to cardiomyogenic cell lines in vitro.

Stamm et al [2003] have proved in a clinical setting that the transplantation of $AC133^+$ stem cells can restore perfusion in ischemic myocardium.

Some authors follow another therapeutic principle, which is TMLR in combination with gene therapy. Lutter et al [Lutter 2002, Heilmann 2003] have shown in studies of chronic myocardial ischemia with a porcine model that the synergistic action of TMLR and vascular endothelial growth factor 121 or fibroblastic growth factor resulted in enhanced arteriogenesis and corresponded with restoration of regional contractility.

These 2 cases demonstrate for the first time the clinical outcome after transepicardial implantation of the highest




Magnetic resonance images obtained preoperatively (A and B) and postoperatively (C and D). Midventricular short-axis view in patient 1 in the end diastolic (A, C) and end systolic (B, D) phase of the cardiac cycle. Note the increased end systolic wall thickness in the anterolateral wall from 6 mm to 8 mm (arrowhead) and from 5 mm to 12 mm (arrow) with significant improvement of wall motion on cine-mode analysis. Improved wall motion with increased end systolic wall thickness could also be detected in the septum and the posterolateral wall (stars in B and D).

number of AC133$^+$ stem cells ever used up to now. The concept of our study was to use the synergistic therapeutic effect of TMLR and stem cell application in terms of neoangiogenic reaction. The inflammation is expected to boost the survival and differentiation of transplanted stem cells in hibernating myocardium.

Rather than a high number of unselected MNCs, we used well-characterized AC133$^+$ cells because of their high potential for multiplication and angiogenic differentiation [Pesce 2003]. We developed a method of intraoperative isolation within less than 3 hours of AC133$^+$ cells of high purity and quantity. To our knowledge our technique provides the highest number of AC133$^+$ cells (up to $9 \times 10^6$) ever transplanted for regeneration of postinfarction myocardium [Stamm 2003]. Our method also allows the surgeon to apply stem cells intraoperatively in emergency cases.

The functional analysis of the myocardial performance, using echocardiography and MRI, revealed dramatic improvement of wall motion at the site of TMLR and stem cell application. Because the TMLR method does not enhance myocardial contractility [Schneider 2001, Lutter 2002, Heilmann 2003] and neither of our patients showed angiographic evidence of major collaterals to the ischemic region, we assume the synergistic effect of stem cell– and TMLR-induced angiogenesis; however, our assumption is of a speculative nature. We think that TMLR in combination with stem cell transplantation might become a novel revascularization therapy for ischemic myocardium.

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